63rd Annual

Drosophila Research Conference

April 6–10, 2022

ABSTRACT BOOK

GENETICS GSA G3 Genes | Genomes | Genetics

9 Origins of adult organ plasticity: How cell lifecycles define tissue states *Lucy O'Brien*, Y.-H. Su, J Liang, S Ngo, E.N. Sanders, H.-T. Sun, A Galenza, P Moreno-Roman, J.W. Millington, A Sherlekar Stanford University

An animal's long-term survival requires that its organs adapt to unpredictable environmental changes. Yet how do mature organs sense external change and deploy an appropriate, reversible response? We seek the cellular and molecular basis of adult organ plasticity—i.e., the remarkable facility with which organs adaptively switch between states of renewal, remodeling, and repair. Our focus is the adult Drosophila gut, whose deep playbook of adaptive responses is executed by a multi-fated collective of stem and terminal daughter cells.
As the cell is the fundamental unit of an organ, we posit that the cell's lifecycle—from birth to differentiation, maturity, and finally death—is the fundamental unit of an organ state. Using new techniques we devised to image functioning guts in live animals, we are resolving these surprisingly diverse lifecycles in real time. We have demonstrated the basis of conservative lifecycle kinetics that maintain the status quo, such as when the gut sustains steady-state turnover by 'closing the loop' between apoptosis and death through the intimate relay of a transient, spatially delimited EGF. When ingested toxins raze large swaths of differentiated cells, the damaged cells unleash cytokines that restore the status quo by concerted acceleration of stem cell divisions and daughter cell differentiation. We also have discovered non-conservative lifecycles that adapt the status quo to dietary change. For instance, when food is withdrawn, the gut quickly shrinks as mature cells abort their lifecycles and forego apoptosis to eject live from the tissue. Altogether, our findings illustrate how organ plasticity flows from extrinsically triggered tuning of single-cell lifecycles.

10 Regulating host phospholipid metabolism to fight infection Michelle Bland University of Virginia

Infection and subsequent immune signaling drive synthesis and secretion of immune effectors and mobilization of immune cells, processes that are supported and directed by host metabolism. In the *Drosophila* larval fat body, the metabolic challenge of fighting infection must be balanced with the energetically-demanding processes of growth and nutrient storage that this organ coordinates. We find that Toll signaling in larval fat body disrupts whole-animal growth and directs a lipid metabolic switch from fat storage to phospholipid synthesis. Although reduced triglyceride storage in larvae with active Toll signaling impairs the response to stress later in life, elevated phospholipid synthesis in fat body supports humoral immune function. Toll signaling in larval fat body directs increased production of membrane phospholipids that coincides with expansion of the endoplasmic reticulum. Phospholipid synthesis in larval fat body supports antimicrobial peptide secretion and promotes bacterial clearance. Our results define a metabolic role for the pleiotropic Toll signaling pathway, and they demonstrate that the demands of fighting infection are met with trade-offs in growth and energy storage to sustain immune function.

11 The genetics basis of inviability in hybrids between *Drosophila melanogaster* and *D. santomea* Daniel Matute University of North Carolina

Evolved changes within species lead to the inevitable loss of viability in hybrids. Inviability is also a convenient phenotype to genetically map and validate functionally divergent genes and pathways differentiating closely related species. Here we identify the *Drosophila melanogaster* form of the highly conserved essential gap gene *giant* (*gt*) as a key genetic determinant of hybrid inviability in crosses with *D. santomea*. We show that the coding region of this allele in *D. melanogaster/D. santomea* hybrids is sufficient to cause embryonic inviability not seen in either pure species. Further genetic analysis indicates that *tailless* (*tll*), another gap gene, is also involved in the hybrid defects. *giant* and *tll* are both members of the gap gene network of transcription factors that participate in establishing anterior-posterior specification of the dipteran embryo, a highly conserved developmental process. Genes whose outputs in this process are functionally conserved nevertheless evolve over short timescales to cause inviability in hybrids.

12 Tissue Biology of Chromosomal Instability Marco Milan IRB Barcelona

Chromosomal Instability (CIN), defined as an increased rate of changes in chromosome structure and number, is a feature of most, if not all, solid tumors. While CIN promotes the gain of oncogene-carrying chromosomes and the loss of tumor-suppressor-gene-carrying chromosomes in certain cancers, its impact on the biology of the cell and on the homeostasis of the tissue, as well as its role in tumorigenesis, are far from being fully elucidated. Our lab has generated an epithelial model of CIN in Drosophila, where the generation of highly aneuploid karyotypes drives cell delamination and cell death. Upon apoptosis inhibition, aberrant karyotypes promote a cell autonomous epithelial to mesenchymal (EMT)-like cell fate transition associated with a highly invasive behavior and the entry into a senescence-like state. I will present our most recent data on the genetic identification and functional characterization of the molecular mechanisms underlying these cellular behaviors and the leading role of aneuploidy in driving these behaviors.

13 Collective Action for Institutional Transformation Shaila Kotadia Stanford University School of Medicine

Within higher education institutions and the sciences is a lack of support, care, and safety for marginalized communities due to a history of systemic oppression. In order to change this, we need to take justice-based approaches for our diversity, equity, and inclusion work, including bringing people together to reenvision culture rather than hundreds of

individual efforts. In decentralized institutions, which are common in higher education, we often do not connect across local (for example, departments, divisions, or units) efforts nor connect local to central administration where decision making occurs. By coming together and breaking down silos, we can gain power in a grassroots manner to lead to culture change across the organization. I will speak on how we can use collective action for institutional transformation using my efforts at Stanford University's School of Medicine Human Resources Group as examples, specifically the efforts of the School of Medicine Staff JEDI (Justice, Equity, Diversity, and Inclusion) Collective. This model demonstrates one method as to how we can move from individual allyship to group co-conspiratorial work to challenge the institution for transformative change. I will also share my personal and professional journey to JEDI work, including transitioning away from Drosophila research to administrative staff JEDI positions.

14 Strategies at UCSF for addressing barriers in science that disproportionately affect people from marginalized groups. Todd Nystul UC San Francisco

A major challenge for the scientific community is that structural inequities in our society create barriers to success in science that disproportionately affect people from currently and historically marginalized groups. These inequities include institutional policies and practices that favor nonmarginalized groups, socioeconomic factors, and societal norms that perpetuate historical biases. They are pervasive, encompassing individuals' experiences both inside and outside of the scientific community, and are often invisible to those who align with or benefit from these systems. The result is that our processes for identifying and promoting individuals based on merit fall short, and thus the scientific community cannot reach its full potential. Because these inequities have multiple causes, a sustained and multifaceted set of strategies are needed to address them. UCSF has a long history of valuing diversity and pursuing efforts to promote and advance justice, equity, and inclusion. I will discuss our recent efforts to increase the diversity of our graduate student population, and to cultivate and sustain a learning and training environment in which students from all backgrounds are able to thrive. Specifically, I will describe the establishment of our new postbaccalaureate program, PROPEL, which aims to provide trainees from historically marginalized backgrounds with the research experience and career mentorship needed to be competitive for top-tier biomedical science PhD and MD/PhD programs; the holistic review process we use for graduate program admissions; and new initiatives to strengthen mentor-mentee relationships in a diverse community. In addition, I will discuss the process we are using to engage students, faculty, and administrators in the development of these initiatives as well as the methods we are using to assess their efficacy. We know that creative solutions to address structural inequities are being developed at many different institutions around the country, and we are eager to share ideas and learn from one another.

15 NINDS Strategies for Enhancing the Diversity of Neuroscience Researchers Marguerite Matthews National Institute of Neurological Disorders and Stroke

The National Institute of Neurological Disorders and Stroke (NINDS) is committed to the development of a biomedical research workforce that is representative of the diversity in American society. NINDS seeks to promote diversity in all of its training and research programs and to increase the participation of individuals from underrepresented populations. The Office of Programs to Enhance Neuroscience Workforce Diversity coordinates NINDS's diversity activities, spanning the training pipeline from innovative neuroscience education outreach (grades K-12) to funding opportunities and important mentoring networks across critical career transition points (from undergraduate up to junior faculty). Some of the funding mechanisms that will be discussed in this session include the following: fellowships for graduate students and postdoctoral trainees, diversity supplements that add funds to an existing grant for mentoring and training individuals from high school to faculty stage, career development awards to assist transition to independent research careers, institutional research education awards to enhance professional development and career advancement of diverse researchers. The strategy to enhance the diversity of neuroscience researchers at NINDS includes interventions that connect programs across critical transition points and activities to assist trainees with the professional development skills and scientific networks to achieve their goals.

16 A conserved anoctamin regulates olfactory neuron firing in *Drosophila* *Pratyajit Mohapatra*, Karen Menuz University of Connecticut

Insects are vectors of several diseases affecting millions of people globally. They interact with their environment primarily by recognizing and distinguishing a plethora of odors. These odors are recognized by odor receptors in the dendrites of olfactory neurons. Insect odor receptors are classified into 2 broad families: Odorant receptors (Or) and Ionotropic receptors (Ir). These 2 families are widely studied and well characterized. However, most individual receptors are poorly conserved across insect species, making them unsuitable candidates for the development of insect repellents. In a previous bioinformatics study, we identified many evolutionarily conserved genes whose expression is highly enriched in the *Drosophila* antenna relative to other tissues. Here, we investigate the role of one of these genes, the anoctamin AnoA. The anoctamin family contains many transmembrane proteins, some of which serve as calcium-gated chloride channels. Our work using a transgenic reporter line indicates that AnoA is broadly expressed in all classes of olfactory neurons; it is also found in hygrosensory and thermosensory neurons. Using epitope tagging, we find that

AnoA protein is specifically localized to the olfactory dendrites. Investigation of AnoA mutants with *in vivo* extracellular electrophysiological recordings reveals that it limits the neuronal firing response to odorants. Together, our data indicate that AnoA plays a critical role in odorant signaling in olfactory neurons, and its expression in the antenna of other insects suggests its function is likely to be evolutionarily conserved.

17 Dissection of the BMP-activated synaptic gene network identifies dichotomous BMP-responsive elements regulating synaptic functions *Robin Vuilleumier*[1], Mo Miao[1], Sonia Giro[1], Clara-Maria Ell[2], Stephane Flibotte[1], Tianshun Lian[1], Annie Collins[1], Sophia Ly[1], George Pyrowolakis[2], Pejmun Haghighi[3], Douglas Allan[1] 1) Department of Cellular and Physiological Sciences, University of British Columbia, Vancouver, British Columbia, Canada.; 2) BIOSS, Centre for Biological Signaling Studies and Institute for Biology I Faculty of Biology, Albert-Ludwigs University of Freiburg, Freiburg, Germany.; 3) Buck Institute for Research on Aging, Novato, CA 94945, USA.

Members of the Transforming growth factor beta (TGF-β)/Bone morphogenetic protein (BMP) family play critical roles in the regulation of synaptic growth, stability, neurotransmission, homeostasis and plasticity of several neuronal subtypes; yet how TGF-β/BMP signaling controls and coordinates these functions remains mostly unknown. Here, we uncovered a neuronal gene network, enriched for essential neurotransmission genes, that is directly controlled by retrograde BMP signaling in Drosophila motor neurons, by combining Drosophila genetics with differential RNA-seq, computational discovery of Smad-binding *cis*-regulatory motifs (*BMP-activating elements*, *BMP-AE* and *BMP-silencer elements*, *BMP-SE*), and reporter analysis of enhancer activity. Surprisingly, we discovered that the *BMP-SE* motif mediates BMP-dependent upregulation of neuronal gene transcription, similar to *BMP-AE*s. Exploring the underlying mechanism for this atypical activity, we found that absence in motor neurons of Shnurri (*shn*), a Smad transcriptional corepressor, switches the repressive function of the *BMP-SE* motif to an activator. This mechanism is underpinned by a lack of *brinker (brk*) repression by retrograde BMP signaling in motor neurons; thus *brk*-dependent de-repression is not a mechanism for BMP gene activation in these neurons. Finally, genome editing of identified *BMP-SE* and *BMP-AE* motifs in the loci of *bruchpilot (brp*) and a novel gene, *without maturity (witty)*, showed that BMP signaling operates through these motifs to regulate synaptic maturation. Taken together, our data broaden the concept of BMP enhancer and silencer motifs, and demonstrate how the retrograde BMP signaling pathway regulates synaptic function.

18 γ-secretase promotes postsynaptic development through the cleavage of a Wnt receptor Lucas Restrepo, Alison DePew, Elizabeth Moese, Stephen Tymanskyj, Michael Parisi, Michael Aimino, Juan Duhart, Hong Fei, *Timothy Mosca* Department of Neuroscience, Thomas Jefferson University, Philadelphia, PA USA

Developing synapses mature through the recruitment of specific proteins that stabilize pre- and post-synaptic structure and function. Wnt ligands signaling via Frizzled (Fz) receptors play many crucial roles in neuronal and synaptic development but whether and how Wnt and Fz influence synaptic maturation is incompletely understood. We demonstrate that Fz2 receptor cleavage via the γ-secretase complex is required for postsynaptic development and maturation. In the absence of γ-secretase, *Drosophila* neuromuscular synapses fail to recruit postsynaptic scaffolding and cytoskeletal proteins, leading to behavioral deficits. Strikingly, introducing *γ-secretase* mutations linked to familial early-onset Alzheimer's Disease into flies leads to synaptic maturation phenotypes that are identical to those seen in null alleles. This conserved role for γ-secretase in synaptic maturation and postsynaptic development highlights the importance of Fz2 cleavage, identifies its mechanism, and suggests that receptor processing by proteins linked to neurodegeneration may be a shared mechanism affecting aspects of synaptic development.

19 Copia, a Drosophila retrotransposable element, regulates structural synaptic plasticity at the larval neuromuscular junction *Peter M'Angale*, Adrienne Lemieux, Alfred Simkin, Cong Xiao, Vivian Budnik, Travis Thomson University of Massachusetts Chan Medical School, Worcester, MA

Despite advances in elucidating the genome, the function, of much of the genetic material, the so called "junk DNA", remains largely unknown. A large portion of junk DNA is comprised of genomic parasites, known as transposable element (TEs). New evidence suggests that a domesticated TE, *activity-regulated cytoskeleton-associated protein 1* (*Arc1*), serves as a mechanism to transport RNAs across the synapse. Here we show that a TE, Copia is enriched at the Drosophila NMJ and Copia is transported across synapses in extracellular vesicles (EVs). We observe Copia^{gag} acting antagonistically to dArc1. Strikingly, downregulation of Copia^{gag} at the Drosophila NMJ results in abnormal NMJ development and increased plasticity. From RNA-sequencing data coupled with digital PCR, we determined that copia is encoded by ~40 different loci each of which is found at different abundances in the Drosophila larval brain or body wall muscles. To our knowledge, this is the first report documenting a physiological role of a TE at synapses, lending further weight to recent arguments and data suggesting that TEs and potentially other types of "junk DNA" are not junk after all.

20 Chromatin regulatory networks underlying coordinated synaptic gene expression *James Kentro*, Erica Larschan, Kate O'Connor-Giles Brown University, Providence, RI

The cellular diversity of the nervous system underlies its ability to respond to stimuli and produce complex behaviors.

Neurons form intricate circuits that transmit information by passing signals through synaptic connections. We have found that hundreds of genetically encoded proteins, both common (pan-neuronal) and neuron subtype-specific, that form the structural and functional components of synapses share a temporal pattern of expression that suggests coordinated regulation. However, no mechanism coordinating the expression of these distinct classes of synaptic genes within and across neuronal subtypes is known. Using motif discovery and analysis of chromatin binding data, we have identified a candidate gene regulatory network that may coordinate expression of synaptic genes. DEAF1, linked to intellectual disability and seizures in humans, and pioneer factors GAF and CLAMP are enriched for binding at synaptic genes and are known to interact with chromatin remodelers and other co-repressor or co-activator proteins. In the *Drosophila* nervous system, we observe a correlation between the timing of initial synaptic gene transcription and substantial increases in chromatin accessibility at synaptic gene promoters that supports a mechanism of coordination through control of the chromatin environment. In *clamp* null mutants, we find an upregulation of synaptic gene transcription. RNAi-mediated knockdown of DEAF1, driven by *pros*-Gal4 in immature neurons, results in increased synaptic bouton number at neuromuscular junctions. These data indicate repressive roles for CLAMP and DEAF1, which have been found to physically interact. The pioneer factor GAF has been found to co-regulate many genes in opposition to CLAMP or DEAF1 in other tissues, and may perform a similar role to increase expression of synaptic genes. We predict this gene regulatory network coordinates expression of pan-neuronal and subtype-specific synaptic genes upstream of terminal selectors by controlling chromatin accessibility at synaptic genes to allow the development of shared traits across distinct neuronal fates.

21 Fatty acid flux through triacylglycerol regulates neuroblast proliferation during oxidative stress *Eva Islimye*, Victor Girard, Andrew Bailey, Alex P. Gould The Francis Crick Institute, London, UK

During development, multipotent neural stem cells proliferate within a specialised local microenvironment called the niche. Work in *Drosophila* and mammals has shown that lipid metabolism is important for regulating the divisions of neural stem cells. During *Drosophila* development, the proliferation of stem/progenitor cells is more protected from hypoxia and oxidative stress in the central nervous system (CNS) than in other tissues. We previously found that the biosynthesis of lipid droplets (LDs) in glia of the neural stem cell niche functions to protect against lipid peroxidation — not only in the glia themselves but also in neighbouring neural stem cells (neuroblasts). Using labelled fatty acids and cell type-specific genetic manipulations, we now investigate the origin of the lipid cargo in the triacylglycerol (TAG) core of glial LDs. We present evidence that oxidative stress triggers neurons to release fatty acids via a process that requires functional mitochondria. Extracellular fatty acids are then taken up by glia and directed towards the TAG core of LDs. Diacylglycerol acyltransferase 1 (Dgat1/Mdy) and adipose triglyceride lipase (Atgl/Bmm) manipulations demonstrate that fatty acid flux through the TAG compartment of glia sustains neuroblast proliferation during oxidative stress. In contrast, fatty acid flux through the TAG compartment of neuroblasts inhibits proliferation during oxidative stress. The contrasting functions of TAG metabolism in glia and neuroblasts are consistent with a model whereby oxidative stress triggers neurons to release potentially toxic fatty acids that are then preferentially captured and processed in niche glia in order to safeguard neuroblast proliferation. Together, our findings reveal how lipid metabolic crosstalk between the three major cell types of the developing CNS functions to spare neural proliferation during oxidative stress.

22 Neuronal activity induces Glucosylceramide that is extruded via exosomes upon glial BMP signals for lysosomal degradation in glia *Liping Wang*[1, 4], Guang Lin[2, 4], Zhongyuan Zuo[2, 4], Hugo Bellen[1, 2, 3, 4] 1) Program in Developmental Biology, Baylor College of Medicine, Houston, TX 77030, USA.; 2) Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX 77030, USA.; 3) Department of Neuroscience, Baylor College of Medicine, Houston, TX 77030, USA.; 4) Jan and Dan Duncan Neurological Research Institute, Texas Children›s Hospital, Houston, TX 77030, USA.

Autosomal recessive variants in *GBA1* cause Gaucher disease (GD), the most prevalent form of lysosome storage disease. *GBA1* encodes a lysosomal enzyme that hydrolyses glucosylceramide (GlcCer) into glucose and ceramide. Loss of *GBA1* leads to increased levels of GlcCer which causes lysosomal dysfunction. We generated a *Gba1b* null allele (*Gba1b*$^{T2A\text{-}Gal4}$) using CRISPR technology. We show that *Gba1b* is specifically expressed in glia and not in neurons at all stages of development as well as in adults. Loss of *Gba1b* causes a significant reduction in lifespan and a progressive activity-dependent loss of synaptic activity of neurons, which is rescued by glial specific expression of human *GBA1*. Moreover, glial but not neuronal specific knockdown of *Gba1b* recapitulates the defects found in *Gba1b* mutants, indicating that *Gba1b* is necessary and sufficient in glia to support neuronal function. We show that GlcCer is synthesized upon neuronal activity and that it is transported from neurons to glia through exosomes. In the absence of *Gba1b*, the glial cells accumulate GlcCer which in turn causes vacuolization and the demise of glia and is followed by neuronal death. The transfer of GlcCer from neurons to glia is induced by a factor secreted by glia. We identified this factor as a BMP ligand, TGF-β, for vertebrate cells. Finally, we show that the White protein, an ABCG transporter, plays an important role in pigment glia and that it modulates GlcCer trafficking to the glial lysosome for degradation by *Gba1b* through the endolysosomal pathway. Based on our observations the White protein is involved in the degradation of GlcCer in parallel with Gba1b.

23 Sequential addition of neuronal temporal cohorts generates a stimulus on-set detection circuit Ellie Heckscher[1, 2, 3], Yi-wen Wang[1, 2, 3], Chris Wreden[1, 2, 3], Maayan Levy[2, 3, 4], *Zarion Marshall*[1] 1) University of Chicago, Chicago, IL, 60637; 2) Institute of Neuroscience; 3) Department of Molecular Genetics and Cell Biology; 4) Department of Neurobiology

Neuronal stem cell lineages are fundamental developmental units of complex brains and neuronal circuits are the fundamental functional units. Understanding how circuits self-assemble starting from neuronal stem cells stem cells is still a largely un-answered developmental question with implications for neuroscience, evolution, and medicine. We use the Drosophila larval nerve cord as a model to study motor circuit assembly. Motor circuits are responsible for processing somatosensory stimuli and generating movement. In prior work, we found that neurons in a single lineage can be organized into groups called “temporal cohorts”. A temporal cohort is a set of neurons that are born during a small time window from a single stem cell and that have shared circuit level function (Wreden, et al., 2017, Meng, et al., 2019, Meng, et al., 2020). In this study, we ask how do neurons from different lineages wire with each other?

As a model, in segment A1 of the Drosophila larval nerve cord, we studied the NB3-3 lineage and the Even-skipped Lateral (EL) interneurons it produces. First, we used Twin-spot MARCM to determine the birth order and morphology of each EL. Then, we mined the Drosophila larval connectome to identify all major direct synaptic inputs onto ELs. Using graph theory, we found that early-born and late-born ELs get significantly different inputs. This supports the idea that early-born and late-born ELs are two temporal cohorts, as was proposed by Wreden et al., 2017. We next characterized the development and function of the circuit containing early-born ELs. early-born ELs get a majority of their input from just four classes of neurons: Chordotonal sensory neurons, Basin interneurons, Ladder interneurons, and EL interneurons segments other than A1. Anatomically, early-born ELs are embedded in a feed-forward motif, getting and functionally they act as on-set detectors for vibrational stimuli. Finally, we determined the developmental origins (e.g., stem cell parent and birth timing) of Ladders and Basins. Basins are a mid-late temporal cohort from the NB3-5 neuroblast, and Ladders are the only temporal cohort from the MNB neuroblast. Basins and Ladders synapse onto early-born ELs, and are born after early-born ELs. Together these data show: 1. Additional lineages are organized into temporal cohorts. 2. There is selective wiring among temporal cohorts. 3. Post-synaptic interneurons are born after their pre-synaptic interneuron partners. These data support a model in which a somatosensory stimulus on-set circuit develops by sequentially assembling a small subset of genetically pre-specified of temporal cohort units.

24 Partial overlap between inversions and genomic islands of divergence during early stages of ecological speciation in *Drosophila yakuba* *Erina A. Ferreira*[1,2], Cathy C. Moore[3], David Ogereau[1], Arnaud Suwalski[2], Héloïse Bastide[1], Jean R. David[1], Rebekah L. Rogers[3], Amir Yassin[1,2] 1) Laboratoire Évolution, Génomes, Comportement et Écologie, CNRS, IRD, Université Paris-Saclay, Gif-sur-Yvette, France; 2) Institut Systématique Evolution Biodiversité (ISYEB) CNRS, MNHN, Sorbonne Université, EPHE, Paris, France; 3) Department of Bioinformatics and Genomics, University of North Carolina, Charlotte, NC

During the early stages of speciation, genetic differences tend to accumulate more rapidly at certain regions of the genome leading to the formation of genomic islands of divergence (GIDs). However, this pattern that has been observed in a wide range of organisms may either be due to selection on beneficial alleles contained within the GIDs and/or difference in the rate of recombination due to structural variation (e.g., inversions, heterochromatic content, etc.). Here, we investigate the possible causes of GIDs in *Drosophila yakuba mayottensis*, a subspecies of *D. yakuba* specializing on toxic noni (*Morinda citrifolia*) fruits on the island of Mayotte. We have previously described this specialization based on a population from a single locality collected in 2013, and identified multiple GIDs through genome comparison with mainland generalist *D. y. yakuba* populations. In 2017, we recollected flies from Mayotte and found *D. y. mayottensis* to be present in three localities, always on noni fruits, reconfirming its strong association with the toxic plant. We sequenced the genomes of the three populations in pools as well as from individual isofemale lines. Comparing these sequences to mainland genomes, we identified 7 GIDs. We assembled a new genome for *D. y. mayottensis* using a combination of Illumina short-read and Oxford Nanopore long-read sequencing methods. We identified 10 genomic regions with major chromosomal rearrangements. Three of those inversions (on the X, 2L and 2R chromosomal arms) overlapped with the GIDs. Experimental evolution using four replicate populations issued from crosses between *D. y. mayottensis* and *D. y. yakuba* showed that the three inversions were likely involved in adaptation on noni, suggesting a partial role of the genomic architecture in host shift. Interestingly, no chromosomal inversions have been detected in *Drosophila sechellia*, the only other *Drosophila* species known to be specialist on noni in the Seychelles archipelago. Despite some similarities at the genic level that we have previously described between the two noni-specialist species, our results indicate that distinct genomic architectures underlie convergent response to common selective pressures in independent lineages.

25 Chromatin Architecture Constrains Where Inversion Breakpoints Occur Over a Short-Time Scale in *D. pseudoobscura* *Dynisty Wright*, Stephen Schaeffer The Pennsylvania State University

DNA in the nucleus is nonrandomly organized into chromosomal territories, compartments, and topological associated domains or TADs. Chromosomal rearrangements have the potential to reorganize this structure, which could potentially

lead to altered gene expression. In humans, genome rearrangements can lead to altered phenotypes, genetic diseases, and cancer due to the misregulation of genes. Unlike in humans, paracentric inversions in *Drosophila* are quite frequent because they do not have the negative effects associated with the formation of unbalanced gametes because males do not recombine, and in females, aberrant meiotic products are lost in polar bodies. As a result, *Drosophila* populations can harbor extensive gene arrangement variation in populations. However, it is not clear whether natural selection acts to remove genome rearrangements because breakpoints alter gene expression because of changes in chromatin architecture. Liao *et al.* (2021) found that TAD structure is conserved over long- evolutionary time between *D. melanogaster* and *D. pseudoobscura*. We used the recent inversion events within *D. pseudoobscura* to determine if chromatin architecture (TAD structure) constrains where breakpoints occur over short-evolutionary time. *D. pseudoobscura* has an abundance of different gene arrangements on the third chromosome. Several hypotheses have been proposed for how new mutations are established in populations including the position effect hypothesis that suggests that inversion breakpoints produce variation for selection to act. We test whether the third chromosome breakpoints alter TAD structure in *D. pseudoobscura* and whether these disruptions altered gene expression. To determine whether breakpoints are constrained, we used seven pairs of inversion breakpoints in *D. pseudoobscura* to determine whether they disrupt TADs or fall between adjacent TADs using the TAD maps of Liao *et al.* (2021). Five of the 13 breakpoints occur at TAD boundaries, which is unlikely due to chance, while eight breakpoints disrupt TADs. We used RNA-Seq data from Fuller *et al.* (2016) to determine if there are differentially expressed genes in disrupted TADs. Of the eight disrupted TADs, seven had differentially expressed genes. Some breakpoints alter gene expression when TADs are disrupted supporting the hypothesis that position effects may contribute to inversion establishment by generating variation for selection to act.

26 Cis-regulatory Changes at the Fatty Acid Elongase *eloF* Underlie the Evolution of Sex-specific Pheromone Profiles in *Drosophila Prolongata* *Yige Luo*[1], Ayumi Takau[2], Takashi Matsuo[2], Santiago Ramirez[1], Artyom Kopp[1] 1) University of California, Davis; 2) Tokyo University, Tokyo

Binary communication systems involving signaling and signal perception play a key role in sexual selection and in the evolution of sexually dimorphic traits. The selective forces and genetic changes underlying such traits can be studied in systems where sex-specific signaling and perception have emerged recently and show signs of coevolution. A promising model is found in *Drosophila prolongata*, which exhibits a species-specific increase in the number of male gustatory bristles. We find that this transition coincides with a change in cuticular hydrocarbon (CHC) profiles. *D. prolongata* males show increased amounts of the long-chained 9-pentacosene (9P) and 9-heptacosene (9H), and a coincident decrease in the shorter-chained 9-tricosene (9T), compared to females. This is in contrast with the closest relatives of *D. prolongata*, in which these CHCs are sexually monomorphic. Perfuming *D. prolongata* females with 9P or 9H reduces copulation success, suggesting that they act as sex pheromones. To unravel the genetic mechanism responsible for evolutionary transition from sexually monomorphic to dimorphic production of these mono-alkenes, we sequenced the oenocyte transcriptomes from males and females of *D. prolongata* and its relative *D. carrolli*. The top candidate gene that emerged from this analysis is *elongaseF* (*eloF*), which encodes an enzyme responsible for fatty acid elongation. *eloF* shows elevated expression in *D. prolongata* males, consistent with the male-specific enrichment of long-chain CHCs in this species. Male-biased *eloF* expression in *D. prolongata* appears to be associated with a species-specific insertion of a transposable element (TE) at this locus. We are currently testing this hypothesis using chimeric reporter constructs that carry a combination of *D. prolongata* and *D. carrolli* sequences. Our results suggest that pheromone synthesis may have coevolved with chemosensory perception and suggest a possible molecular mechanism for the evolution of male-female communication.

27 Dissecting the genetic changes underlying the adaptation of the carbon dioxide receptor in the *D. suzukii* species complex *Alice Gadau*[1], Xin Yu Zhu Jiang[1,2], Sylvia Durkin[1], Nicolas Svetec[1], Li Zhao[1] 1) The Rockefeller University; 2) CUNY-Hunter College

Adaptation is fundamental to the survival and reproduction of living organisms. However, the genetic and neural basis underlying adaptation to new environments remains largely unknown. Novel traits allow species to invade new ecological niches and habitats. Pest species therefore provide a unique opportunity to study adaptation as they exploit new environments. One pest species, *Drosophila suzukii*, has evolved a preference to oviposit in ripe fruits instead of rotten fruit, unlike most *Drosophilids*. The *Drosophila* sensory and central nervous system may contribute to adaptive behavioral shifts. To determine candidate genes that contribute to the adaptive shift in *D. suzukii*, we identified sensory genes under directional selection in *D. suzukii* and showed differential expression between *D. suzukii* and *D. biarmipes*, *D. subpulchrella*, and *D. melanogaster* using population genetic analysis and RNA-seq analysis. From this screen, 15 genes evolved under positive selection and were significantly differentially expressed between species. One candidate, the carbon dioxide receptor *Gr63a*, is of particular interest as its sequence has diverged in D. *suzukii* yet is expressed significantly more in *D. subpulchrella*. We hypothesize that CO_2 is playing a role in decision-making in the *D.suzukii* species complex. By conducting a two-choice preference trial for high and low CO_2 concentration, we found that *D. suzukii* and *D. subpulchrella* exhibited the same indifference to CO_2, which is significantly different from *D. melanogaster'*s aversion. In

addition, we found that the CO_2 sensing neuron is more sensitive to CO_2 in *D. suzukii* and *D. subpulchrella* compared to *D. melanogaster*, using single sensillum electrophysiology. These data suggest that the behavior is conserved between the two species and that the evolution of *Gr63a* may have occurred at the regulatory region in *D. subpulchrella* yet the gene sequence in *D. suzukii.* To test this hypothesis, we use a GAL4-UAS system to dissect the role of regulatory and protein changes of *D. subpulchrella* and *D.suzukii*. Our results provide novel insight into the link between novel genotypes and phenotypes, and the complexity of the evolution of novel behavior.

28 Faster-X: Evolution of *Drosophila melanogaster* and *Drosophila simulans* Sex-biased Expression and Associated Chromatin *Adalena Nanni*[1,2], Natalie Martinez[1], Rita Graze[3], Sarah Signor[4], Srna Vlaho[5], Sergey Nuzhdin[5], Rolf Renne[1,2], Lauren McIntyre[1,2] 1) Department of Molecular Genetics and Microbiology, University of Florida, Gainesville, FL; 2) University of Florida Genetics Institute, University of Florida, Gainesville, FL; 3) Department of Biological Sciences, Auburn University, Auburn, AL; 4) Department of Biological Sciences, North Dakota State University, Fargo, ND; 5) Department of Biological Sciences, University of Southern California, Los Angeles, CA

Antagonistic coevolution between the sexes should drive the faster evolution of reproductive-related characteristics, including sexually dimorphic morphology and behavior. Interestingly, the closely related human commensal species, *D. melanogaster* and *D. simulans*, have diverged in sexual dimorphic behaviors. These behaviors are potential targets of sex antagonism. While trends of sex-biased expression tend to be tissue-specific in Drosophila, there has been more male-biased expression observed in head and brain tissues, with an enrichment of male-biased genes on the X chromosome. However, the relationship between sex-biased expression and chromatin in the context of sex dimorphism and evolution is not well understood. Here we show evidence for faster evolution of the X for both chromatin and gene expression in *D. melanogaster* and *D. simulans*, with underlying sex-biased genes conserved between the species. We observe more sex-biased expression, specifically male-biased expression, on the X compared to the autosomes in both species. Additionally, male open chromatin marks are enriched on the X. Open chromatin marks in each of the sexes are associated (70% concordant) with sex-biased expression in the corresponding sex. These results indicate chromatin plays a role in sex dimorphism of expression and evolution.

29 Phage-derived DNAses are novel innate immune cell effectors in animals *Kirsten Verster*[1], Gyongyi Cinege[2], Zoltan Lipinszki[2], Lilla Magyar[2], Rebecca Tarnopol[1], Eva Kurucz[2], Istvan Ando[2], Noah Whiteman[1] 1) University of California - Berkeley, Berkeley, CA; 2) Szeged Biological Research Centre, Szeged, Hungary

Symbiotic mutualisms with microbes have played an important role in the evolution of phenotypic novelties in animals. One of the most striking examples is a protective mutualism between sap-feeding insects such as the pea aphid (*Acyrthosiphon pisum*) and the intracellular bacterial symbiont *Ca.* Hamiltonella defensa. Some strains of *Ca.* H. defensa acquired a lysogenic bacteriophage called *A. pisum* Secondary Endosymbiont (APSE) that encodes diverse bacterial toxins. APSE phages protect insect hosts against parasitoid braconid wasp attack by killing wasp eggs with toxins in the haemocoel. We discovered *cytolethal distending toxin B (cdtB)* genes encoding CdtB, a widespread DNAse I and virulence factor, were horizontally transferred at least five times from APSE phages to insect nuclear genomes. In *Drosophila ananassae*, in addition to *cdtB*, we found two copies of *cdtB* fused to another apoptosis-inducing toxin gene, *apoptosis inducing protein 56 (aip56)*. We found that expression of these phage-transferred insect *cdtB* and *cdtB::aip56* fusion genes is upregulated in insect fat body and immune cells (hemocytes) after wasp parasitism. Loss-of-function *cdtB* and *cdtB::aip56* mutant lines of *D. ananassae* were more susceptible to wasp attack than wild-type flies and show phenotypic differences in sexual maturity and development time. Our results show how potent effectors can be co-opted through symbioses to become integral components of animal immune systems.

30 Widespread introgression across a phylogeny of 155 *Drosophila* genomes *Anton Suvorov*[1], Bernard Kim[2], Jeremy Wang[3], Ellie Armstrong[2], David Peede[3], Emmanuel D'Agostino[3], Donald Price[4], Peter Wadell[5], Michael Lang[6], Virginie Courtier-Orgogozo[6], Jean David[7,8], Dmitri Petrov[2], Daniel Matute[3], Daniel Schrider[1], Aaron A. Comeault Comeault[9] 1) Department of Genetics, University of North Carolina, Chapel Hill, North Carolina. USA; 2) Department of Biology, Stanford University, Stanford, CA, USA; 3) Biology Department, University of North Carolina, Chapel Hill, North Carolina. USA; 4) School of Life Sciences, University of Nevada, Las Vegas. USA; 5) School of Fundamental Sciences, Massey University, Palmerston North. New Zealand; 6) CNRS, Institut Jacques Monod, Université de Paris. France; 7) Laboratoire Evolution, Génomes, Comportement, Ecologie (EGCE) CNRS, IRD, Univ. Paris-sud, Université Paris-Saclay, Gif sur Yvette, France; 8) Institut de Systématique, Evolution, Biodiversité, CNRS, MNHN, UPMC, EPHE, Muséum National d'Histoire Naturelle, Sorbonne Universités, Paris, France; 9) Molecular Ecology & Evolution Group, School of Natural Sciences, Bangor University, Bangor, Gwynedd LL57 2DGA, UK

Genome-scale sequence data have invigorated the study of hybridization and introgression, 28 particularly in animals. However, outside of a few notable cases, we lack systematic tests for 29 introgression at a larger phylogenetic scale across entire clades. Here we leverage 155 genome 30 assemblies, from 149 species, to generate a fossil-calibrated phylogeny and conduct multilocus 31 tests for introgression across nine monophyletic radiations within the

genus *Drosophila*. Using 32 complementary phylogenomic approaches, we identify widespread introgression across the 33 evolutionary history of *Drosophila*. Mapping gene-tree discordance onto the phylogeny revealed 34 that both ancient and recent introgression has occurred across most of the nine clades that we 35 examined. Our results provide the first evidence of introgression occurring across the evolutionary 36 history of *Drosophila* and highlight the need to continue to study the evolutionary consequences 37 of hybridization and introgression in this genus and across the Tree of Life.

31 An odorant binding protein is required for mating plug formation and male fertility in *Drosophila* *Nora Brown*[1], Benjamin Gordon[1], Snigdha Misra[1], Caitlin McDonough-Goldstein[2], Andrew Clark[1], Geoffrey Findlay[3], Mariana Wolfner[1] 1) Cornell University, Ithaca, NY; 2) University of Vienna, Vienna, Austria; 3) College of the Holy Cross, Worcester, MA

In *Drosophila melanogaster* and other insects, the seminal fluid proteins (SFPs) and male sex pheromones that enter the female with sperm during mating are essential for fertility and induce profound post-mating effects on female physiology and behavior. Genes encoding SFPs include some of the fastest evolving in the genome and display remarkable levels of turnover between species, likely the consequence of sperm competition and/or sexual conflict. The SFP suite in *D. melanogaster* includes several members of large gene families, including the odorant binding protein (Obp) family. Previous work in *Drosophila* has shown that some Obp genes are highly expressed in the antennae and can mediate behavioral responses to odorants, potentially by binding and carrying these molecules to odorant receptors. These observations have led to the hypothesis that the seminal Obps might act as molecular carriers for pheromones or other compounds important for male fertility in the ejaculate. Interestingly, Obps are found in the seminal fluid of several arthropod species, though the reproductive functions of these proteins in any species remains uncharacterized. By analyzing comparative RNAseq data from the male reproductive tract of multiple *Drosophila* species, we found that one seminal Obp, Obp56g, shows high male reproductive tract expression only in a subset of species in the *melanogaster* and *obscura* groups, suggesting co-option of this protein for reproductive function over evolutionary time. We used RNAi and CRISPR/Cas9 generated mutants to test the role of Obp56g, as well as the other six seminal Obps, in *D. melanogaster* fertility. Whereas male flies lacking six of the seven seminal Obp genes tested so far were fully fertile, males lacking Obp56g failed to induce post-mating responses in their mates. We found that Obp56g is expressed in the male's ejaculatory bulb, an important tissue in the reproductive tract that synthesizes components of the mating plug. Indeed, we found males lacking Obp56g fail to form a mating plug in the mated female's reproductive tract, which is needed to induce post-mating responses and sperm storage. We then used tissue specific RNAi experiments to show that ejaculatory bulb/duct expression of Obp56g is necessary for both mating plug formation and male fertility. Together, this work uncovers a novel role for Obp56g in reproduction and enhances our understanding of seminal fluid evolution.

32 *A novel role for CRTC linking age-related cardiac dysfunction and fibrosis to metabolic syndrome* *Cristiana Dondi*, Stan Walls, Anais Kervadec, Sean Zeng, Cecilia Hurtado, Karen Ocorr Sanford Burnham Prebys Medical Discovery Institute

Heart disease (HD) is a leading cause of mortality for both men and women, currently accounting for approximately 1 of every 4 deaths in the US. Obesity and diabetes greatly increase the risk of HD, although the underlying genetic and metabolic mechanisms remain unclear. **CRTC**, a **C**REB (cAMP-responsive element binding protein) - **R**egulated **T**ranscription **C**o-activator, has been identified as a "nutrient sensor" and a key regulator of metabolism in mammals and is conserved in *Drosophila.* In the liver, CRTC mediates signaling from glucagon and insulin, however, a cardiac-specific role for CRTC has yet to be identified. Using our *Drosophila* heart model, we found that CRTC null mutants ($CRTC^{-/-}$) exhibit severe cardiac restriction with reduced stroke volume and tachycardia, accompanied by cardiac fibrosis, a hallmark of heart disease. Cardiac-specific knockdown (KD) of CRTC, or its coactivator CREB, mimics the heart defects of $CRTC^{-/-}$. In the liver, CRTC is activated by calcineurin (CN), and in vertebrate hearts CN is known to cause cardiac hypertrophy via activation of NFAT. In the fly, overexpression of CRTC or activated CN, also known as Pp2B, also cause hypertrophy. However, in the fly cardiac KD of NFAT has no effect on function and CRTC knockout (KO) blocks all hypertrophic effect of cardiac- specific overexpression (OE) of Pp2B suggesting that, in the heart, CRTC mediates the downstream effects of CN. We also show that *CRTC3* is highly expressed in zebrafish hearts and KD causes cardiac restriction, as in flies. In both fly and fish, CRTC localizes in the Z-bands as well as in myocardial cell nuclei. CRTC 1, 2 & 3 are also expressed in human iPSC-derived cardiomyocytes (hiPSC-CM) and KD of both CRTC 2&3 caused broadened action potentials, further supporting a fundamental role of CRTC in heart function. Comparative analysis of cardiac gene expression revealed that pathways involved in glucose, fatty acid, and amino acid metabolism were contra-regulated between cardiac CRTC KD and OE flies, suggesting that CRTC acts as a metabolic switch in the heart in regulation of lipid, carbohydrate, and protein metabolism. In summary, we have identified CRTC gene and its orthologues as a novel signaling pathway that maintains heart function with likely relevance linking nutrient sensing to heart disease.

33 glial GBA links neural lipid metabolism and proteostasis with sleep *John Vaughen*[1], Emma Theisen[2], Ina Anreiter[2], Irma Magaly Rivas-Serna[3], Vera Mazurak[3], Thomas Clandinin[3], Tom Clandinin[2] 1) Stanford University, Department of Developmental Biology; 2) Stanford University, Department of Neurobiology; 3) University of Alberta, Department of Agriculture, Food, and Nutritional Science

Lifelong brain health is sustained by macromolecule recycling through autophagy-lysosomal and proteasomal degradation pathways. How brains balance membrane recycling and intensive neurotransmission remains mysterious. To better understood the cellular underpinnings of brain membrane homeostasis, we investigated glucocerebrosidase (GBA), a critical lysosomal protein which hydrolyzes the sphingolipid glucosylceramide (GlcCer) and is commonly mutated in neurological diseases, including Parkinson's. We show that *Drosophila gba1b* brains harbor widespread and heterogeneous proteostasis defects: engorged neural lysosomes appear during pupal development and persist throughout life, whereas ubiquitinated protein aggregates progressively afflict neurons and glia. Using cell-type specific Gba1b manipulations, we demonstrate that glial Gba1b is necessary and sufficient for regulating brain proteostasis. Which glia mediate GBA's wide-ranging effects? Depleting Gba1b in individual glia subsets caused no detectable changes, while depletion in both barrier and ensheathing glia phenocopied panglial *gba1b* knockdown. Interestingly, although Gba1b overexpression in neurons was toxic, placing Gba1b directly under a neural promoter largely rescued *gba1b* null defects. Thus, Gba1b is made by glia but can function in neurons to control brain lipid metabolism and proteostasis.

Given these complex phenotypes, we hypothesized that differential lipid accumulation occurred across time and cell-type. We conducted targeted lipidomic across multiple ages and timepoints from *gba1b* brains. GlcCer species were immediately elevated in young mutant brains, and ectopic GlcCer staining colocalized with neural lysosomes. Additionally and unexpectedly, younger *gba1b* brains harbored certain GlcCer species only at a specific zeitgeber, while these species were always detectable in older brains. We thus re-examined young *gba1b* brains for aggregates across time and found transient aggregates prominently in glia processes in the optic lobe chiasms. We are exploring if this aggregate clearance cycle is under circadian clock control and/or driven by light and neural activity. Do *gba1b* mutants show other circadian-related phenotypes? Using activity assays, we demonstrate that *gba1b* mutants as well as glial *gba1b* knockout flies are hyperactive and sleep less but still retain circadian rhythms when housed in constant darkness. Intriguingly, other short sleeping mutants also harbor ectopic aggregates in optic chiasm glia, hinting at a connection between sleep and glia proteostasis. These studies reveal compartmentalization of sphingolipid lysosomal enzymes in glia and support the hypothesis that membrane recycling via glia is a molecular function of sleep.

34 Macrophages-derived Pvf2 modulates developmental transition by ecdysone synthesis regulation *Sergio Juarez-Carreño*, Frederic Geissmann Immunology program, Sloan Kettering Institute, Memorial Sloan Kettering Cancer Center, NY, NY, USA.

Disturbances during development are sensed by monitoring hormones and signals released from the impaired tissues. All developing tissues and organs have the ability to release and receive information about the growth status of themselves or the different parts of the body. This communication mechanism involves hormones, peptides, and metabolites. Recent evidence suggests that macrophages act as a multi-organ rheostat that constantly senses and monitors homeostasis. Moreover, macrophages have the ability to relay signals, as growth factors, to mitigate perturbations and restore homeostasis during development. Recent studies from my host lab demonstrated that flies lacking macrophages disrupt the developmental transition between juvenile (larva) to puberty (pupa) stages. Developmental timing is regulated by sterols hormones synthesis in endocrine glands. However, how macrophages sense perturbations (such as nutrition) and communicate with the neuroendocrine system to control developmental timing remains unsolved.
In this presentation, we will discuss how macrophages communicate with the neuroendocrine system. First, we proved that the lack of macrophages during development regulates the sterol hormone synthesis (ecdysone) in the prothoracic gland by reducing the Halloween genes transcription. To further understand how macrophages regulate ecdysone synthesis, we carried out a knockdown genetic screen of growth factors secreted by macrophages during development. We obtained Pvf2 as a factor required in macrophages to control developmental timing. Reduction of *pvf2* expression in macrophages induces developmental delay by reducing Halloween genes transcription and ecdysone synthesis. Moreover, the lack of Pvf2 receptor, *pvr*, in the prothoracic gland induces developmental delay by ecdysone production inhibition. In addition, the lack of *pvf2* in macrophages reduces their total number in larva stages, suggesting a mechanism where Pvr monitors the total number of macrophages in the body through Pvf2 before ecdysone pulse turn up for developmental transition in L3-instar larvae. The *pvf2*-depleted macrophages phenotypes are recapitulated in the *pvf2* null mutant. The developmental delay and ecdysone synthesis inhibition in *pvf2* null mutant were rescued by overexpression of *pvf2* in macrophages or *pvr* in the prothoracic gland. Therefore, our data suggest a communicative process between macrophages and the neuroendocrine system involving Pvf2/Pvr to fine-tune ecdysone synthesis in the larva to pupa transition.

35 The *Drosophila* enzyme L-2-hydroxyglutarate dehydrogenase is required in the renal system for recovery from hypoxic stress *Nader Mahmoudzadeh*[1], Yasaman Heidarian[1], Katherine Beebe[2], Alexander Fitt[1], Aylin Rodan[2], Jason Tennessen[1] 1) Indiana University; 2) University of Utah

The oncometabolite L-2-hydroxyglutarate (L-2HG) is considered a waste-product of central carbon metabolism that is capable of disrupting chromatin architecture, mitochondrial metabolism, and cellular differentiation. As a result, ectopic L-2HG accumulation in humans is toxic and promotes the growth of renal cell carcinomas, however, few studies have

examined how this molecule functions *in vivo*. The fruit fly *Drosophila melanogaster* has emerged as a powerful model to study L-2HG. Not only are the metabolic mechanisms that regulate L-2HG accumulation conserved between flies and humans but L-2HG levels undergo predictable fluctuations during the fly lifecycle that allow for mechanistic studies. Here we exploit this system to understand how inappropriate L-2HG accumulation affects the metabolism and physiology of adult flies. Using CRISPR/Cas9, we generated mutations in the sole *Drosophila* L-2-hydroxyglutarate dehydrogenase (L2HGDH) ortholog, which is required to degrade L-2HG. Although *L2hgdh* mutant adults accumulate L-2HG levels that are 50-times higher than controls, these mutants are viable and fertile under normal conditions and exhibit no apparent phenotypes. However, *L2hgdh* mutant adults are extremely sensitive to hypoxic stress and die following exposure to 1% O_2. Subsequent metabolomic studies indicate that hypoxia exposed *L2hgdh* mutants become locked in a glycolytic state and are unable to restart mitochondrial metabolism upon reoxygenation. Finally, we find that the hypoxia-sensitive phenotype of *L2hgdh* mutants stem, in part, from defects in the Malpighian tubules (MTs). Not only do *L2hgdh* mutant MTs display mitochondrial defects, but *L2hgdh* mutants that express a rescuing transgene in only the principal cells of MTs exhibit normal viability following hypoxia exposure. Considering that renal cell carcinomas are the only cancer where *L2hgdh* is known to function as a tumor suppressor, our findings suggest that L-2HG is capable of uniquely disrupting renal cell function and establish the fly as a powerful disease model for studying this oncometabolite.

36 Differential regulation of glycogen homeostasis by TGFβ/Activin ligands *Heidi Bretscher*, Michael O'Connor University of Minnesota- Twin Cities

Maintaining carbohydrate homeostasis is essential for organismal health. Insufficient carbohydrates stores results in an inadequate amount of energy to fuel cellular processes. Conversely, excess carbohydrates leads to metabolic diseases such as type II diabetes. We have found that the TGFβ/Activin signaling pathway is required for homeostasis of stored carbohydrates known as glycogen. In *Drosophila* the TGFβ/Activin signaling family consists of three ligands: Activinβ, Dawdle and Myoglianin. All ligands signal through a single type I receptor, Baboon, however Baboon has three separate splice isoforms that differ in the ligand binding domain. Each ligand signals through a single splice isoform and tissues express a specific splice isoform. Thus, each ligand results in activation of the TGFβ/Activin signaling pathway in a specific subset of tissues. Interestingly, Activinβ is a positive regulator of glycogen storage, whereas Dawdle negatively regulates glycogen stores. This suggests the effect of TGFβ/Activin signaling on glycogen homeostasis depends on the tissue in which the signal is received.
We have found that motorneuron derived Activinβ signals directly to the muscle to positively regulates glycogen storage. Thus, *activinβ* mutants have low levels of glycogen in muscle. Glycogen levels in *activinβ* mutants can be partially rescued by inhibiting glycogen phosphorylase (GlyP), which catalyzes the breakdown of glycogen. Despite increased breakdown of glycogen by GlyP, loss of Activinβ does not result in increased glucose levels suggesting that byproducts of glycogen breakdown are being used to fuel another process.
In addition to serving as a positive regulator of glycogen storage in muscle, Activinβ is also a positive regulator of fat body glycogen levels. Intriguingly, Dawdle negatively regulates fat body glycogen storage, highlighting the tissue specific role of TGFβ/Activin signaling in maintaining glycogen homeostasis. Additionally, loss of Activinβ and Dawdle results in differing responses to a high sugar diet. Loss of Activinβ protects animals from carbohydrate accumulation on a high sugar diet, while Dawdle is required for survival on a high sugar diet.

37 Spenito, m^6A RNA modification and the establishment of metabolic sexual dimorphism in larvae *Arely V. Diaz*, Kelsey Hazegh, Tânia Reis University of Colorado, Anschutz Medical Campus

Metabolism in males and females is fundamentally different. We found dimorphic expression of sex-determining genes in metabolic tissues (the fat body) and investigated the underlying molecular mechanisms. We previously found that Spenito (Nito), an RNA-binding protein and a subunit of the N6-methyladenosine (m^6A) methyltransferase complex, 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. We propose a similar role for Nito in the establishment of metabolic sexual dimorphisms. Fat body-specific Nito knockdown recapitulated the lean phenotype and abolishes fat differences between males and females. To address the requirement of m^6A, we knocked down other members of the complex. Fat body-specific knockdown of three other members of the m^6A complex also made larvae lean, but the differences between the sexes were mostly preserved. At the molecular level, Nito was also required to maintain sex-specific fat body identity: Nito depletion caused mis-splicing of Sxl, a master regulator of the sex determination cascade, masculinizing the expression profile of female fat bodies. Altogether, our findings support a model in which Nito establishes differential expression of target genes in males versus females, leading to sexual dimorphisms in fat storage.

38 Acetyl-CoA mediated autoacetylation of fatty acid synthase as a metabolic switch for *de novo* lipogenesis in developing *Drosophila* *Ting Miao*, Hua Bai Iowa State university, Ames, IA

De novo lipogenesis (DNL) is tightly regulated during animal growth and development. It is well known that under conditions like excess nutrition, obesity, and cancer, DNL is activated through transcriptional regulation of lipogenic genes, including fatty acid synthase (FASN). Surprisingly, we show that the levels of FASN protein remain unchanged

during *Drosophila* larval development, while its acetylation increases in fast-growing larvae and is positively correlated to the FASN enzymatic activity. This finding suggests that the regulation of FASN activity and developmental DNL is not at the transcription and translation steps; rather, it is regulated through post-translational modifications (PTMs), especially lysine acetylation. Through mass-spectrometry analysis, we identified two evolutionarily conserved lysine residues that are acetylated in both fly and human FASN proteins. One of them, K813 in fly, is located nearby the active site of the malonyl/acetyltransferase (MAT) domain. Acetylation of K813 is predicted to enlarge the binding pocket of MAT domain to allow fast substrate loading. Indeed, flies with lysine to arginine substitution (K813R, acetylation deficiency mutants) show decreased lipogenesis and FASN activity, reduced body weight, and delayed pupariation. To further understand how acetylation of lysine residue K813 is regulated, we screen all major lysine acetyltransferases (KATs) and deacetylases (KDACs and Sirtuins). We identified deacetylase Sirt1 as the key negative regulator of FASN acetylation. However, none of the KATs that we examined seems to play a role in promoting the acetylation of K813. Intriguingly, our genetics and biochemical studies show that acetylation of lysine residue K813 does not require the participation of KATs, while it is mediated by acetyl-CoA levels. Through site-specific mutagenesis, we identified three residues downstream of K813 that consist of a new functional motif (R/Q-xx-G-x-G/A) of FASN, which is responsible for acetyl-CoA recognition and binding. Mutations of these residues block the autoacetylation of K813 *in vitro*. Taken together, our results uncover a novel regulation in metabolic homeostasis. It is suggested that the site-specific autoacetylation at lysine residue K813 is crucial in sensing the fluctuated fuels (acetyl-CoA) and fine-tuning FASN enzymatic activity. Thus, our findings discover a novel self-regulatory module that links signaling metabolite acetyl-CoA, lysine acetylation, and DNL.

39 Mechanical activation of mitochondrial energy metabolism during cell differentiation *Zong-Heng Wang*[1], Christian Combs[1], Jay Knutson[1], Yongshun Lin[1], Jizhong Zou[1], Mary Lilly[2], Hong Xu[1] 1) National Heart, Lung, and Blood Institute, Bethesda, MD; 2) National Institute of Child Health and Human Development, Bethesda, MD

Mitochondria affords eukaryotes great metabolic flexibility to balance energy metabolism and cellular homeostasis. Unicellular organisms can adjust metabolic programs response to the availability of nutrients in environment. In multicellular organisms, a metabolic shift from glycolysis to oxidative phosphorylation (OXPHOS) is often observed during the differentiation of various types of stem cells and progenitors. To support proliferation, cells emphasize on glycolysis to preserve carbon sources for biosynthetic pathways. Post-mitotic cells utilize OXPHOS, a more effective way producing ATP, to power cellular activities. However, the cellular processes orchestrate this metabolic transition are largely unknown. We previously demonstrated that mitochondrial biogenesis and OXPHOS are activated in the differentiating ovarian cyst in *Drosophila*. Here, we report that mechanical forces generated by the surrounding somatic cells trigger JNK signaling and mitochondrial energy metabolism in differentiating germ cells. Somatic cells compress the differentiating cyst. Using fluorescence lifetime imaging microscopy (FLIM), we found that compression increases the membrane tension of germ cells. Abolishing somatic cells' engulfment by genetically inhibiting Notch signaling, relieves the membrane tension on germ cells and blocks the activation of OXPHO activity as well. We carried out candidate RNAi screen on genes involved in mechanosensation and identified that the stretch-activated ion channel (SAC), Tmc, is required for OXPHOS activation. In compressed differentiating cysts, gating of Tmc maintains cytosolic Ca^{2+} levels, which induces the transcriptional activation of OXPHOS through a CaMKI-Fray-JNK signaling relay. Moreover, our preliminary data show that SACs mediate contraction-induced OXPHOS during the maturation of human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs), suggesting that mechanical activation of mitochondrial energy metabolism is evolutionarily conserved among animals.

48 The septate junction protein Bark beetle (Bark) is required for *Drosophila* intestinal barrier function and homeostasis *Rachel Hodge*[1], Martin Resnik-Docampo[1], Emma Edmond[1], Fernando de la Torre[1], Cecilia D'Alterio[1], D. Leanne Jones[1,2] 1) University of California Los Angeles, Los Angeles, CA; 2) University of California San Francisco, San Francisco, CA

In the intestine, the epithelial barrier is maintained by tight junctions (TJs) in mammals and septate junctions (SJs) in insects. The intestinal barrier allows para-cellular flow of water, ions and nutrients across the epithelium, while maintaining food matter and microbes inside the intestinal lumen. Age-related loss of intestinal barrier function has been found across multiple species, including *Drosophila melanogaster* and humans. The age-related causes of barrier dysfunction remain unknown. The tricellular junction (TCJ) is a specialized region of the SJ where three adjacent cells meet. Previous studies by our lab indicated that mis-localization of the TCJ protein Gliotactin (Gli) is correlated with aging. Depletion of Gli in young flies leads to loss of intestinal homeostasis, including increased intestinal stem cell (ISC) proliferation, a hallmark of aging. In the embryonic epithelium, the TCJ protein Bark is required to recruit Gli to the TCJ. Therefore, we hypothesized that Bark would be required for maintenance of intestinal homeostasis and barrier function, similar to Gli. Indeed, depletion of Bark from the TCJ of enterocytes in a young fly posterior midgut (PMG) led to an increase in ISC proliferation, accelerated age-associated intestinal barrier loss, and shortened lifespan, suggesting Bark is required for maintenance of intestinal homeostasis. Antibody staining for Gli and Bark shows a decrease in intensity at the TCJ in intestines from aged flies, with a modest, but significant, increase in Bark staining at the BCJ and in the cytoplasm. Previous RNAseq data indicated that expression of SJ protein genes, including Bark, is not decreased with age,

and overexpression of Bark does not rescue age-associated loss of barrier function. Unlike in the embryonic epithelium, depletion of Gli disrupted proper localization of Bark to the TCJ in the PMG. In summary, the TCJ protein Bark is required at the TCJ to maintain intestinal homeostasis and barrier integrity in *Drosophila.* Our work on the mechanisms leading to loss of the intestinal barrier will provide insight into strategies to treat age-related gastrointestinal diseases, such as cancer.

49 Role of Intramembrane Spastic Paraplegia Proteins in Organization of Axonal ER and ER-mitochondria Contacts in Drosophila *ZEYNEP OZTURK*, Holy ROBERTSON, Joe STONE, Shuler XU, Cahir O'KANE University of Cambridge, Department of Genetics, Cambridge, UK

The Hereditary Spastic Paraplegias (HSPs) are a group of rare, clinically and genetically heterogeneous, inherited neurodegenerative and neurodevelopmental diseases characterised by spasticity and lower limb weaknesses. More than 80 causative genes are known, and some of them imply the importance of endoplasmic reticulum (ER) -**a neuron within a neuron**- function and morphogenesis in HSPs. These HSP proteins are Spastin (SPG4), Atlastin (SPG3A), Receptor Expression Enhancing Protein 1 (REEP1/SPG31) and Reticulon (SPG12), which share a common feature of one or two intramembrane hairpin domains that can recognise or drive curvature of ER membrane. Proteins of the REEP and reticulon families appear to be responsible for forming most peripheral ER tubules in yeast. In Drosophila, removing these families leads to fewer ER tubules in axons, of wider diameter, although there is no widespread absence of tubules. Therefore, other proteins must be involved in shaping the tubular ER network in Drosophila axons. Another HSP protein with predicted hairpin domains is C19orf12; this is therefore another candidate protein for contributing to shaping the axon ER network. Mutations in this gene are found in patients with autosomal recessive HSP and Neurodegeneration with Brain Iron Accumulation (NBIA). C19orf12 protein colocalises with mitochondria and ER, and with ERmitochondria contacts. Most C19orf12 mutations are found in predicted transmembrane (TM) regions. To investigate possible roles of C19orf12 in ER and mitochondria structure and function, we have generated loss-of-function mutants of the widely expressed Drosophila ortholog of C19orf12, CG3740, using P element excision and CRISPR/cas9. These mutants are homozygous viable, as are quadruple mutants lacking CG3740 and all the widely expressed reticulon and REEP proteins, suggesting that these 4 proteins together are not sufficient for tubular ER formation. Testing of ER and mitochondria morphology in these mutants is in progress. For this purpose, we have also generated flies carrying a split-GFP reporter for ER-mitochondrial sites. To identify additional candidate proteins for ER shaping, we have also performed bioinformatic analyses of proteins shown by proteomic analysis to be enriched on ER tubules. These analyses have identified most of the known proteins with roles in ER shaping, implying that it may also be a good way to identify additional proteins with similar roles.

50 The Abelson tyrosine kinase cooperates with the Nedd4-family ubiquitin ligase Suppressor of Deltex to regulate the late endosomal passage of Notch and modulate signaling activation *Julio Miranda-Alban*, Nicelio Sanchez-Luege, Xiao Sun, Fernando Valbuena, Benjamin Glick, Ilaria Rebay University of Chicago

The conserved Notch signaling pathway controls a plethora of cellular processes during development and tissue homeostasis across animal species, and abnormal signaling activity of Notch has been linked to several developmental disorders and pathologies. The Notch protein is a transmembrane receptor and cleavage of Notch to release its intracellular domain (NICD) allows the latter to enter the nucleus and enforce the transcriptional output of signaling. NICD cleavage is best studied in response to receptor-ligand interactions but can also occur in the absence of ligand. As cells continuously traffic Notch receptor from the cell surface into endocytic compartments regardless of ligand presence, release of the NICD at different steps of the endocytic pathway is possible. Thus, Notch endocytic trafficking offers a unique opportunity to finely-tune signaling output. In this context, a key regulator of the endosomal passage of Drosophila Notch is the Nedd4-family ubiquitin ligase Suppressor of Deltex (Su(dx)), which recognizes a PPxY motif within the NICD to promote Notch internalization from the membrane of late endosomes/multivesicular bodies (MVBs) into their luminal space. This prevents signaling activity by both topologically restricting the release of the NICD into the cytoplasm if cleaved and promoting the subsequent lysosomal degradation of Notch.
In this study we have used the pupal wing and S2 cultured cells to uncover a role for the non-receptor tyrosine kinase Abelson (Abl) in the late endosomal passage of Notch. We find that *loss of abl* in both models results in an aberrant accumulation of Notch at the membrane of late endosomes due to compromised internalization into the lumen of these compartments, and this de-represses signaling. Moreover, Abl performs this role in a kinase-dependent manner that requires the PPxY motif of the NICD, the same motif used by Su(dx) to promote internalization of Notch into the late endosomal lumen. As endocytic trafficking is a substantial contributor to the extraordinary variety of routes for Notch activation, careful and robust regulation of Notch at each step of this transport is critical for preventing non-physiological signaling activity. Our study offers important new mechanistic insight into how two post-translational modifiers, a kinase (Abl) and a ubiquitin ligase (Su(dx)), converge upon the same molecular motif (NICD PPxY) to confer robustness to the modulation of Notch activity during late steps of its endosomal passage.

51 Understanding the Role of Loner in Myoblast Fusion. *Amrita Shrikant Gokhale*, Donghoon Lee, Peter Keene, Elizabeth Chen UT Southwestern Medical Center

Cell-cell fusion is a fundamental process in the development of multicellular organisms, where it is involved in diverse processes such as fertilization, myogenesis, bone resorption and placenta formation. *Drosophila* embryonic musculature is an excellent genetically tractable model to study cell-cell fusion, as it results from the fusion between two types of cells – muscle founder cells and fusion competent myoblasts (FCMs). Previous studies in our lab have identified many molecular components in myoblast fusion and revealed an asymmetric "fusogenic synapse", wherein the FCM generates an actin-enriched podosome-like structure (PLS) to invade the founder cell to promote cell membrane fusion.
We previously identified an Arf GTPase guanine nucleotide exchange factor (ArfGEF), Loner, as an essential regulator of myoblast fusion. However, its precise molecular mechanism of action remains poorly understood. We found that actin-enriched PLS form at the fusogenic synapse between founder cells and FCMs in *loner* mutant embryos. However, time-lapse imaging data suggest that they are more diffused and unstable than the control embryos, failing to invade the founder cells properly to induce cell fusion. Our genetic rescue experiments showed that while Loner in required in both founder cells and FCMs, it plays a major role in the FCMs. Consistent with this, Loner is recruited to the fusogenic synapse in the FCMs but not the founder cells. Our structure-function analysis of Loner show that multiple domains of Loner can interact with FCM-specific cell adhesion molecule Sns, suggesting that Loner may be recruited to the fusogenic synapse by Sns. Thus, we hypothesize that Loner promotes actin nucleation promoting factors at the fusogenic synapse of FCMs. Currently, we are investigating how Loner and its downstream Arf GTPases interact and regulate the actin nucleation promoting factors.

52 Pericentrin-Like-Protein is a Kinesin-1 Adaptor that drives Centriole Motility. *Matthew Hannaford*, Rong Liu, Neil Billington, Zachary Swider, Brian Galletta , Carey Fagerstrom, James Sellers, Nasser Rusan National Heart Lung and Blood institute, NIH, Bethesda, MD

Centrosomes are a key microtubule organizing center in the cell. They comprise a pair of centrioles surrounded by a matrix of proteins termed the pericentriolar material. Through microtubule (MT) nucleation centrosomes organize the mitotic spindle, cilia and flagella. To fulfill these functions, centrosomes must be motile to achieve proper positioning within the cell. Very little is understood about the different mechanisms of centrosome motility. Typically, motility is thought to be governed by the activity of MT motors, pushing or pulling on the microtubules anchored at the centrosome. In some cell types, centrioles lack PCM and microtubules, and are referred to as inactive centrioles. Inactive centrioles must be motile as their positioning is critical for asymmetric cell division. Despite this, the mechanisms of inactive centriole movement are not well understood. We investigated how inactive centrioles move in interphase cells. High resolution live imaging in Drosophila revealed that centrioles are microtubule cargo and move along the MT network in a manner involving Kinesin-1. Importantly, super resolution imaging demonstrated that Kinesin-1 localizes to the outside of the centriole in interphase cells. An RNAi screen identified Pericentrin-Like-Protein (Plp) as essential for interphase centriole movement. Through yeast-2-hybrid and an in vivo interaction assay we found that Plp interacts with the cargo binding region of the Kinesin-1 heavy chain. *In vitro* analysis showed that Plp and Kinesin-1 comigrate on MTs. Furthermore, autoinhibition of Kinesin-1 inhibits interaction with PLP. Relieving Kinesin-1 autoinhibition using hinge region mutations promotes robust PLP binding. Using random mutagenesis, we generated a series of mutations in Plp which ablate interaction with Kinesin-1, and significantly perturbs centriolar transport. Finally we show that centriole transport is essential for correct centrosome activity and inheritance in asymmetrically dividing neural stem cells. Our data support a model where Plp acts as a novel motor adaptor that links the centriole to the MT transport machinery, facilitating movement. 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.

53 Uncovering the mechanism of BNIP3-mediated mtDNA selection in the female germline *Anastasia Minenkova*[1], Swathi Jeedigunta[1], Jonathan Palozzi[1], Yun Li[1,2], Thomas Hurd[1] 1) University of Toronto, Toronto, Ontario, Canada; 2) The Hospital for Sick Children, Toronto, Ontario, Canada

Mitochondria are essential, intracellular energy-producing organelles that contain their own DNA (mtDNA). In metazoans, mtDNA is subject to high mutation rates, inherited uniparentally, and undergoes little recombination. This makes mtDNA prone to the accumulation of deleterious mutations, which can cause severe disease. To prevent deleterious mutations from being inherited, the female germline has evolved a conserved quality control mechanism, termed mtDNA selection. During this process, mitochondria with deleterious mutations are purged from the germline. The mechanism of mtDNA selection is poorly understood; however, we have recently identified that mitochondrial autophagy (mitophagy) is required for this process in *Drosophila*. We find that the outer mitochondrial membrane mitophagy receptor BNIP3 is required for both selection and mitophagy. BNIP3 mediates the incorporation of mitochondria into autophagosomes through the interaction with the core autophagy machinery component Atg8a through its LC3 interacting region (LIR). BNIP3 also plays a minor role in the *de novo* assembly of autophagosomes, which independent of its LIR. Together, our results suggest that BNIP3-mediated mitophagy represents a generalizable mechanism for selection against deleterious mtDNA mutations, which may enable the development of strategies for the treatment of mtDNA disorders.

54 Tissue-specific chromatin profiling reveals a key role for Clock-dependent transcription in regulation of Drosophila photoreceptor homeostasis *Juan Jauregui-Lozano*, Vikki Weake Purdue University, West Lafayette IN 47907

The chromatin landscape defines cellular identity in multicellular organisms with unique patterns of DNA accessibility and histone marks decorating the genome of each cell type. Further, modulation of the chromatin landscape contributes to the regulation of most nuclear processes, including replication, transcription and DNA repair. Thus, profiling the chromatin state of different cell types in an intact organism under disease or distinct physiological conditions can provide insight into how chromatin regulates cell homeostasis in vivo. The aging eye experiences physiological changes that include decreased visual function and increased risk of retinal degeneration. Although there are transcriptomic signatures in the aging retina that correlate with these changes, the gene regulatory mechanisms that contribute to cellular homeostasis during aging remain to be determined. To identify transcriptional regulatory mechanisms that contribute to the homeostasis of the aging eye, we utilized a tissue-specific approach to profile the transcriptomic and accessible chromatin landscape of *Drosophila* photoreceptor neuron in an aging time course. ATAC-seq and RNA-seq data integration identified 61 transcription factors that showed differential activity in aging Drosophila photoreceptors. These 61 age-regulated transcription factors included two circadian regulators, Clock (CLK) and cycle (CYC), that showed sustained increased activity during aging. When we disrupted Clock activity in adult photoreceptors using a CLK dominant negative mutant, we observed changes in expression of 15 – 20% of genes including key components of the phototransduction machinery and several eye-specific transcription factors. Using ATAC-seq, we showed that loss of Clock activity leads to changes in activity of 31 transcription factors and causes a progressive decrease in global levels of chromatin accessibility in photoreceptors. Supporting a key role for Clock-dependent transcription in the eye, disruption of Clock activity in photoreceptors also induced light-dependent retinal degeneration and increased oxidative stress, independent of light exposure. Since disruption of circadian rhythms has been recently associated with the onset of many age-related eye diseases, our data suggests that during normal aging, the circadian TF complex CLK:CYC protects the aging retina by directing gene regulatory networks that maintain expression of the phototransduction machinery and counteract oxidative stress.

55 Circadian autophagy drives longevity response to Intermittent Time-Restricted-Feeding (iTRF) Matthew Ulgherait Columbia University Medical Center

Time-restricted feeding (TRF) has become an anti-aging treatment of great interest in recent years, with the potential to delay aging and improve health in diverse organisms from *Drosophila* to humans. TRF consists of restricting food intake to specific hours of the day. Because TRF simply controls the timing of feeding rather than nutrient or caloric content, TRF has been hypothesized to depend on circadian-regulated functions including many metabolic functions. Nonetheless, the underlying molecular mechanisms of TRF-mediated metabolic regulation remain unclear. To exploit the rapid genetic tools and well-characterized aging markers of *Drosophila*, we developed an alternate-day, intermittent TRF (iTRF) dietary regimen for flies that robustly extended their lifespan and delayed aging-dependent processes such as poly-ubiquitinated protein aggregation in muscles and intestinal dysplasia. We showed that iTRF treatment enhanced circadian-regulated transcription and that iTRF-mediated lifespan extension required molecular components of both the circadian clock and autophagy, a well conserved longevity pathway. Night-specific induction of autophagy was both necessary and sufficient to extend lifespan on *ad lib* diet and also prevented further iTRF-mediated lifespan extension. In contrast, day-specific induction of autophagy did not extend lifespan. Thus, these results identify “night-specific” circadian-regulated autophagy as a critical contributor to iTRF-mediated health benefits in *Drosophila.* As both circadian regulation and autophagy are highly conserved processes that play roles in mammalian aging, this work highlights the possibility that behavioral or pharmaceutical interventions stimulating circadian-regulated autophagy may provide people with similar health benefits such as delays in aging pathology and lifespan extension.

56 The Neuronal and Molecular Mechanisms by Which Death Perception Impacts Fly Behavior and Lifespan *Tuhin Chakraborty*, Christi Gendron, Cathryn Duran, Scott Pletcher University of Michigan, Ann Arbor

Certain perceptive experiences, such as the sudden death of a companion or repeated exposure to traumatic events, have significant behavioral and physiological effects that are well established across taxa. Recently, we demonstrated that when *Drosophila melanogaster* perceive dead conspecifics in their environment, they exhibit behavioral and physiological changes that influence energy metabolism, stress resistance, and lifespan. Sight and serotonin signaling through receptor 5-HT2A are required for these effects to manifest upon exposure to dead. New data using neural tracing and genetic analyses identified the ellipsoid body, a neuropil in the central complex that is known to be important for multifaceted sensory integration and motor coordination functions, as an important structure underlying the effects of death perception on lifespan. To better understand how this structure transduces perceptual experience into physiological changes, we dissected the contributions of individual ellipsoid body ring neurons and found that activity of the R4d and R4m neurons were required for the effect of death perception on lifespan. To elucidate the molecular mechanisms, we executed a targeted screen and discovered that RNAi-mediated knock down of 5HT2A in R4d neurons abrogated the lifespan effects caused by death perception. We also discovered that FOXO expression, a transcription factor associated with the insulin-signaling pathway and a known regulator of aging, was required in R4d neurons.

Further evidence suggests a role for the insulin pathway in mediating the effects of death perception on lifespan; dilp3 and dIlp5, but not dIlp2, showed increased mRNA levels in dead-exposed wild type flies compared to the unexposed control animals. In addition, this mRNA increase was reflected at the protein level as dIlp3 immunostaining was higher in dIlp-expressing neurons of dead exposed compared to the unexposed controls. At present we are testing a model in which visual input to the ellipsoid body influences insulin production to modulate psychological state, physical health, and aging.

57 Blocking cell fusion inhibits age-induced polyploidy and maintains epithelial organization in *Drosophila* *Ari Dehn*[1], Navdeep Gogna[2], Patsy Nishina[2], Vicki Losick[1] 1) Boston College, Chestnut Hill, MA; 2) The Jackson Laboratory, Bar Harbor, ME

A characteristic of normal aging and age-related diseases is the remodeling of a tissue's cellular organization through polyploid cell growth. Polyploidy arises from an increase in nuclear ploidy or the number of nuclei per cell. However, it is not known whether age-induced polyploidy is an adaption to stressors or a precursor to degeneration. Here, we find that the adult fruit fly's abdominal epithelium becomes polyploid with age through generation of large multinucleated cells that make up more than 40% of the tissue area. The syncytia arise by cell fusion, not endomitosis. Epithelial multinucleation is also a characteristic of macular degeneration, including *Ctnna1*^{tvrm5}, a mouse model for pattern dystrophy. Similarly, we find that the knockdown of alpha-catenin enhances multinucleation in the fly epithelium. We further show that age-induced polyploidy can be suppressed by inhibiting cell fusion revealing a means to maintain tissue organization in older animals.

58 Mechanisms of Systemic and Cellular Growth Control by Cholesterol *Mette Lassen*, Michael J. Texada, Lisa H. Pedersen, Kim Rewitz University of Copenhagen, Denmark

Growth control is fundamentally important for normal biological development, with nutrient availability being a key factor regulating cellular and systemic growth and maturation timing (including mammalian puberty and insect metamorphosis). Cholesterol is an essential lipid used as substrate for steroidogenesis and as a structural component of cellular membranes. Diet is a major source of cholesterol, and adipose tissue is a main cholesterol storage depot, especially in obesity. Emerging evidence indicates that cholesterol is an important regulator of cell growth and thereby plays a critical role in health and disease. In fact, cholesterol has recently been identified as a promoter of the occurrence, metastasis, and mortality of cancers such as breast and prostate cancer and glioblastoma, and this lipid has therefore become a clinically important therapeutic target. Despite this importance, the mechanism by which cholesterol regulates both normal cell growth and cancer development remains poorly understood. Since growth control is a fundamental process during development, the signaling pathways that regulate growth have been conserved between flies and humans. We have found that cholesterol's influence on systemic and cellular growth in *Drosophila* larva arises primarily through effects on the conserved intracellular nutrient sensor Target of Rapamycin (TOR) and the superimposed insulin-like signaling system, the main hormonal system controlling systemic growth. Increasing intracellular cholesterol levels either by dietary supplementation or through knockdown of the lysosomal cholesterol transporter Niemann-Pick Type C-1a (NPC1a) increases TOR activity, leading to increased cell growth. These effects in the cells of the fat body and glia of the blood-brain barrier lead to increased insulin production, release and signaling, whereas in the prothoracic gland, cholesterol-driven TOR activity promotes endoreduplication and thus appears to alter or bypass the "critical weight" checkpoint. Cholesterol induced TOR activity appears to be tightly regulated and exhibits temporal dynamics that differ from those seen in amino acid-induced TOR activity. Dysregulation of the TOR and insulin/IGF signaling systems are frequently linked with cancers, and thus these findings may further our understanding of the links between cholesterol, hormonal signaling, and growth control in cancer development, as well as and the connection between childhood obesity and early puberty.

59 Hypoxia-dependent Control of Larval Maturation *Michael Turingan*, Tan Li, Savraj Grewal University of Calgary

When exposed to low oxygen (hypoxia), *Drosophila* larvae can survive by reducing their growth rate and delaying development to the pupal stage. While the mechanisms that mediate reduced growth have been described, it is less clear how hypoxia delays maturation from the larval to pupal stage. This maturation is controlled by a neuroendocrine circuit in which neuronal projections from the brain innervate the prothoracic gland (PG), an endocrine organ that produces the steroid hormone ecdysone. Activation of this circuit at the end of the larval period triggers a pulse of ecdysone to initiate maturation. We find that larvae reared in hypoxia (5% oxygen) from hatching show delayed ecdysone production (as evidenced by delayed induction of ecdysone biosynthetic genes) resulting in a ~2-day delay in larval maturation. Although hypoxic larvae are delayed in their attainment of critical weight (CW), switching larvae to hypoxia after the CW checkpoint still led to a strong developmental delay, suggesting that hypoxia can specifically act on the larval-to-pupal transition. Interestingly, our data suggest that the developmental delay seems to be independent of the classic hypoxia regulator, HIF-1α and nutrient/TOR signaling. Instead, we find a role for alteration of Ras/MAPK signaling. Expression of ecdysone biosynthetic genes, and thus ecdysone production, is controlled by the conserved Ras/MAPK signaling pathway. PG-specific activation of this pathway is sufficient to reverse the hypoxia-induced developmental

delay. Upstream of this MAP kinase pathway in the PG are multiple receptor tyrosine kinases (RTKs). One of these - Epidermal Growth Factor Receptor (Egfr) - has been reported as a major controller of ecdysone production at the end of the larval period. PG-specific RNAi-mediated knockdown of Egfr causes a marked developmental delay. In hypoxic larvae, however, Egfr knockdown in the PG does not delay development any more than hypoxia alone, suggesting that hypoxia and Egfr-knockdown are working in the same pathway. Moreover, Egf ligands, spitz and vein, which mediate autocrine Egfr signaling in the PG, show a delayed induction in hypoxic larvae. We thus find that the post-CW maturation defect in hypoxia may involve suppressed Egfr/Ras signaling in the PG. Owing to conservation in key aspects of steroid hormone regulation, this work adds to our understanding of whole-body responses to hypoxia during development.

60 The role of Jagunal protein in the establishment of cortical polarity in Drosophila melanogaster neuroblast *Lelahiwat Legesse*, Blake Riggs San Francisco state university

In the central nervous system (CNS), cell diversity is accomplished by asymmetric cell division, a fundamental and highly conserved process during development of multicellular life. Drosophila melanogaster neural progenitor cells, or neuroblasts (NB), are one of the best models for studying asymmetric division. Neuroblast undergoes asymmetric division generating one selfrenewing daughter neuroblast and another cell known as a ganglion mother cell (GMC). An essential factor for asymmetric division is the correct establishment of cellular polarity which is required for the correct partitioning of cell fate determinants. However, it is unclear the pathway by which these determinants are distributed and arranged during asymmetric divisions. Apicalbasal polarity is established through an evolutionarily conserved protein complex that includes Bazooka (Baz), Par-6, aPKC. Previous research has determined that the endoplasmic reticulum (ER) divides asymmetrically during mitosis prior to neuroblast differentiation with the requirement of a novel ER protein Jagunal (Jagn). Here we propose to investigate if ER asymmetry during mitosis plays a role in cortical polarity. Through genetic cross and immunohistochemistry, I determined that in the larval neuroblast, ER does not co-localize with apical or basal cell fate determinates, Bazooka (Baz) or Prospero (Pros), respectively. Future studies will include embryo fixations to investigate if there could be a possible colocalization earlier in development as well examining if Baz is necessary for ER asymmetric partitioning during cell division. These approaches will help define the role of Jagn in asymmetric ER division and neuroblast development.

61 Exploring the role of dynein in transporting *cen* mRNA to the centrosome *Hala Zein-Sabatto*[1], Li Jin[2], Simon L. Bullock[2], Dorothy Lerit[1] 1) Emory University Atlanta, GA, USA; 2) MRC Laboratory of Molecular Biology Cambridge, UK

The centrosome is a multi-functional organelle that plays a key role in nucleating and organizing microtubules, facilitating ciliogenesis, and organizing the bipolar mitotic spindle during cell division. A protein matrix known as the pericentriolar material (PCM) surrounds the centrosome and regulates centrosomal function. Various mRNAs also concentrate around the centrosome, but their functional significance is not yet fully understood. Our lab and others have identified *centrocortin* (*cen*) mRNA as forming micron-scale RNA granules near the centrosome in a cell cycle-dependent manner. We also showed localization of *cen* mRNA at the centrosomes is needed for error-free mitosis. However, how *cen* mRNA localizes to the centrosome is still unknown. Preliminary data from immunofluorescent imaging combined with smFISH reveals *cen* mRNA decorates astral microtubules. Further, biochemical studies show that Cen protein, a component of the *cen* mRNA granule, interacts with the dynein motor complex. Taken together, these data suggest that *cen* mRNA is transported to the centrosome via dynein-directed trafficking along microtubules. To test this hypothesis, *cen* mRNA localization was quantified in CRISPR-edited *Drosophila* embryos designed to disrupt a predicted dynein light intermediate chain binding site within the *cen* sequence. Similarly, *cen* mRNA localization was quantified in embryos of classical dynein heavy chain transheterozygous mutants. Moreover, the effect of destabilizing the microtubule network on *cen* granule localization to the centrosome was analyzed. Inhibiting the interaction of *cen* mRNA with dynein and microtubules resulted in a decrease in *cen* mRNA granules localized to the centrosome, consistent with a role for dynein in RNA trafficking to this site. Furthermore, fly lines harboring N- and C-terminal truncations of the *cen* transcript were made to identify the minimal sequence required for *cen* mRNA localization to the centrosome. This work provides insight into the dynamic localization of *cen* mRNA through active intracellular transport.

62 Elucidating the mechanism of coactivator Taiman/AIB1-driven cell competition and its relation to the Adenomatous Polyposis Coli (APC) tumor suppressor in *Drosophila* *Colby Schweibenz*, Ken Moberg Emory University

The phenomenon of 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 (EcR), are able to kill wild type 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 disc to test and confirm the reciprocal hypothesis, that cells with reduced Tai expression (Tai^{low}) are competitive 'losers,' and we use a genetic screen in the adult eye to identify candidate mechanisms required for elimination of these cells by wild type neighbors. This screen recovered 'hits' in the pro-apoptotic genes, *head involution defective*, *reaper,* and *grim*, as dominant suppressors of the loss of Tai^{low} cells, confirming a competitive mechanism that operates through the classic DIAP-RHG-Caspase apoptotic pathway. In addition to these validating 'hits', we also recovered alleles of factors involved in cell:cell signaling pathways that are thus candidates to act downstream of Tai to control competitive fate. Our studies have focused on two of these: 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 (Suijkerbuijk, 2016). We find that Apc1/Apc2 loss rescues elimination of Tai^{low} cells in both eye and wing epithelia, and couple this with evidence that Tai controls some Wg-target genes in larval wing cells. We will describe ongoing experiments to establish mechanistic links between Tai and Wg/Apc in the wing epithelium, with the goal of defining how the Tai/EcR and Wg/Apc pathways intersect to determine winner/loser status in *Drosophila* epithelia.

63 Cell-surface proteomic profiling identifies key regulators in epithelial cell competition *Ke Li*[1,2], Juan Oses-Prieto[2], Alma Burlingame[2], Lily Yeh Jan[1,2], Yuh Nung Jan[1,2] 1) Howard Hughes Medical Institute; 2) University of California, San Francisco

Cell competition defines an evolutionarily conserved, cell-cell interaction mechanism by which more fit cells ("winners") eliminate and replace suboptimal cells ("losers"). One classic example is wild-type cells eliminating the adjacent cells bearing mutated or reduced ribosomal protein genes (*Rp*), emphasizing the significance of the cell-surface interaction between competing populations. Despite recent progress in identifying intracellular molecular events underlying cell competition, little is known regarding how distinct cell populations recognize and compare their relative fitness. Moreover, although mechanical forces have been proposed to regulate cell competition, the components mediating mechanical responses remain elusive. Given the particular interests in the competing interface, we labeled proteins on winner and loser cell surfaces in a spatiotemporal-specific manner and created a quantitative profile using a multiplexed proteomics approach. Using clonal knockdown of *Rp* in imaginal epithelia, we find ~250 and ~500 proteins are significantly enriched in winner and loser surface proteomes, respectively. Consistent with previous findings, we find autophagy-related proteins are enriched in loser cells, whereas engulfment-related proteins are overrepresented in winner cells. Remarkably, our proteomics data indicates wild-type cells have lower levels of non-muscle myosin expression compared to cells with reduced *Rp*. Wild-type cells indeed have reduced junctional tension, and restoring junctional tension suppresses cell competition. Interestingly, mechanosensitive channels are highlighted in the winner cells, coincident with their higher calcium signaling. Our results further demonstrate that mechanical stress is required for cell competition, identifying candidate genes mediating mechanical sensing. Efforts are currently underway to perform a proteome-instructed screen to identify novel regulators of cell competition and these results will also be presented.

64 Hypoxia-dependent regulation of epithelial tissue growth and development *Abhishek Sharma*, Savraj Grewal Department of Biochemistry and Molecular Biology, University of Calgary, Canada

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 *tsc* and *foxo* function in the developing larval imaginal disc epithelial tissues. Cell clones mutant for both *tsc1* and *foxo* show an overgrowth phenotype under normal growth conditions. We find that this overgrowth is exacerbated under hypoxia and leads to altered tissue patterning. Moreover, we also find that *tsc1/foxo* double mutant 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. In contrast to loss of *tsc1/foxo* we see that clones overexpressing oncogene *yorkie* do not show enhanced growth under hypoxic conditions. We are currently exploring mechanisms by which hypoxia exposure enhances the overgrowth of *tsc1/foxo* mutant tissue. 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.

65 Late Endosomes act as carriers for delivery of Ceramide phosphoethanolamine (CPE) with unique acyl chain anchors to cleavage furrows during male meiosis cytokinesis. *Govind Kunduri*[1], Si-Hung Le[2], Baena Valentina[3], Nagampalli Vijaykrishna[4], Daniel Blankenberg[4], Yoshihiro, Izumi[2], Takeshi Bamba[2], Kedar Narayan[3], Usha Acharya[1], Jaira Acharya[1] 1) Cancer and Developmental Biology Laboratory, Center for Cancer Research, National Cancer Institute; 2) Department of Metabolomics, Kyushu University; 3) Center for Molecular Microscopy, Center for Cancer Research, National Cancer Institute; 4) Genomic Medicine Institute and Lerner Research Institute, Cleveland Clinic, Cleveland

Cell division, wherein one cell divides into two daughter cells, is fundamental to all living organisms. Cytokinesis, the final step of cell division, begins with the formation of an actomyosin contractile ring, positioned midway between the segregated chromosomes. Constriction of the ring with concomitant membrane addition in a spatiotemporal manner generates a cleavage furrow that physically separates the cytoplasm. Unique lipids with specific biophysical properties have been shown to localize to midbodies, however, their delivery mechanisms and biological roles remain largely unknown. In this study, we show that Ceramide phosphoethanolamine (CPE), the structural analog of sphingomyelin, has unique acyl chain anchors in spermatocytes and is essential for meiosis cytokinesis. The head group of CPE is also important for spermatogenesis. We found aberrant central spindle and contractile ring behavior but not de-localization of phosphatidyl inositols (PIPs) at the plasma membrane was responsible for the male meiosis cytokinesis defect in CPE deficient animals. Further, we demonstrate the enrichment of CPE in Rab7 and Rab11 positive endosomes which in turn translocate to the cleavage furrows to promote cytokinesis. Volume electron microscopy analysis using Correlative light and focused ion beam scanning electron microscopy showed that CPE enriched Rab7 positive endosomes appear as giant, interconnected multivesicular bodies that are juxtaposed on contractile ring material. Genetic ablation of Rab7 alone results in significant meiotic cytokinesis defects. Our results imply endosomal delivery of CPE to ingressing membranes is crucial for male meiotic cytokinesis.

66 Immunostimulatory Lipids in Drosophila Bacterial Infection *Sophia Parks*[1], Susan Nguyen[1], Daiki Fujinaga[2], Naoki Yamanaka[2], Adler Dillman[1] 1) Department of Nematology, University of California, Riverside, California; 2) Department of Entomology, University of California, Riverside, California

Eicosanoids are C20 polyunsaturated fatty acid (PUFA) derivatives that carry out many essential roles in mammalian and insect systems including development, reproduction, and immunity. Eicosanoids such as prostaglandins and leukotrienes are synthesized from arachidonic acid that is cleaved directly from the cell membrane. Insects, however, have been shown to have low levels of C20s in circulation and it has been hypothesized that eicosanoids are synthesized from C18 precursors such as linoleic acid. Here we show that *D. melanogaster* exhibits higher levels of C18s in the hemolymph and that these are depleted after a bacterial infection. These depleted oxylipinds as well as certain downstream prostaglandins are able to rescue the outcome of infection. This work identifies oxylipins that are involved in immunity and supports the notion that *D. melanogaster* utilizes immunostimulatory and immunosuppressive lipid signaling mechanisms to mitigate bacterial infections. Our understanding of immune signaling in the fly and its analogies to the mammalian system will allow for an even more detailed use as a model organism in immune studies.

67 Interorgan transfer of intact micron-sized lipid droplets to macrophages during the Drosophila immune response *Ishneet Kaur*, Amber Myers, Eniola Ogundipe, Catherine Brennan Cal State University Fullerton

Macrophages are white blood cells of the immune system in both mammals and Drosophila. In addition to their roles in engulfing and killing microbial invaders by phagocytosis, these cells play important roles in embryonic development, tissue repair, metabolism, and maintenance of homeostasis. Under certain inflammatory conditions, mammalian macrophages accumulate lipids in the form of lipid droplets, including in infection, atherosclerosis, and obesity; however, neither the mechanisms nor purpose of this accumulation are well-understood. We have found the fly macrophages [plasmatocytes] also accumulate lipid droplets, with astonishing speed following bacterial infection: filling up to 30% of cell volume within 3 hrs of infection. We have discovered a completely novel mechanism for this inflammatory lipid accumulation: instead of synthesizing lipids from fatty acids or glucose taken up from the blood, fly macrophages obtain intact, micron-sized lipid droplets from the fat body, through a phagocytosis-like mechanism we term “phagoliposis”. By blocking the metabolism of these lipid droplets, we have discovered some unexpected inflammatory mechanisms fueled by these lipids.

68 Paying the amino acid cost of the humoral immune response to bacterial infection *William H. Pearson*[1], Krista L. Grimes[1], Ashima D. Wadhawan[1], Jake Jacobson[1], Esteban J. Beckwith[2], Gerald Larrouy-Maumus[1], Marc S. Dionne[1] 1) Centre for Molecular Bacteriology and Infection, Imperial College London, UK; 2) Instituto de Fisiología, Biología Molecular y Neurociencias, UBA-CONICET, Buenos Aires, Argentina

Immunity and metabolism are closely intertwined. Immune responses are resource-demanding and metabolic changes are therefore required to the supply the immune system with the required energy and raw materials. In pathological infections where the immune response becomes dysregulated, these metabolic changes can contribute to pathology. Work using the model organism *Drosophila melanogaster* has been productive in uncovering how the immune system regulates metabolism, but details of actual metabolic changes that occur during infection, and their functions, are poorly known.
This project seeks to uncover the metabolic changes that occur during the *Drosophila* innate immune response to infection and understand the consequences of these changes. By combining mass spectrometry-based metabolomic analysis with genetic approaches, we found that successful immune responses to systemic bacterial infection caused an increase in nitrogenous waste metabolism. Further investigations suggested that this increase in nitrogenous waste is due to upregulation of endogenous protein degradation via an ESCRT-III mediated process. Expression of *CHMP2B*,

encoding a core ESCRT-III component, is indicated to be required in fat body for the optimal translation of antimicrobial peptide transcripts. Together these data suggest a model in which the fly upregulates degradation of its own protein in order to rapidly provide in-demand amino acids for immune effector production, with catabolism of the resulting excess amino acids causing an increase in nitrogen waste metabolism. This project has thus revealed a profound metabolic change induced by a successful immune response in *Drosophila*. As the cost of this change is the degradation of the host's own protein, this a probable mechanism of metabolic pathology in the context of a dysregulated immune response. Studying the etiology of such metabolic pathology will contribute to our understanding of conditions underpinned by metabolic dysregulation, such as sepsis and infection-induced cachexia.

69 The cytoplasmic incompatibility factor proteins CifA and CifB are both nucleases in *Drosophila melanogaster* *Rupinder Kaur*[1,2], J. Dylan Shropshire[3], Brittany Leigh[1,2], Seth Bordenstein[1,2,4,5] 1) Department of Biological Sciences, Vanderbilt University, Nashville, TN, USA; 2) Vanderbilt Microbiome Initiative, Vanderbilt University, Nashville, TN, USA; 3) Division of Biological Sciences, University of Montana, Missoula, MT, USA ; 4) Department of Pathology, Microbiology & Immunology, Vanderbilt University Medical Center, Nashville, TN, USA; 5) Vanderbilt Institute for Infection, Immunology and Inflammation, Vanderbilt University Medical Center, Nashville, TN, USA

The widespread symbiosis between invertebrates and *Wolbachia* bacteria is due in part to cytoplasmic incompatibility (CI) that is significant to arthropod vector control and evolution. While CI selectively favors the fitness of *Wolbachia*-transmitting females and thus the bacterial spread, the CI mechanism is not broadly understood. In *Drosophila melanogaster*, dual expression of the genes *cifA* and *cifB* from *w*Mel causes CI, resulting in embryonic lethality between symbiotic male and aposymbiotic female cross. These two co-diverging genes span five phylogenetic Types, and previous work suggested different CifB Types are distinguishable by the presence/absence of nuclease activity. Here, we re-evaluate this claim using transgenic, mutant, enzymatic, and cytochemical assays *in vitro* and *in situ*. We demonstrate that contrary to prior conclusions, T1 CifB is an *in vitro* DNase that cleaves single- and double-stranded DNA. Moreover, we provide the first characterization of T1 CifA as a nuclease that cleaves both DNA and RNA. We further show that *in vitro* DNase activity translates to *in situ* spermatid DNA fragmentation in Cif-expressing and *Wolbachia*-bearing testes, and CifB-mediated spermatid DNA damage alone is insufficient to cause CI. Cif enzymatic activity is ablated by deletions and amino acid substitutions. In sum, these molecular insights (i) highlight previously unrecognized nuclease functions of T1 CifA and CifB proteins (ii) reinforce a conservative gene nomenclature and mechanistic model that is agnostic to presumptions about distinct Cif enzymatic activities and (iii) support a postulate of the Host Modification model of CI that spermatid DNA is altered by the Cif proteins.

70 A symbiotic niche in the Drosophila gut regulates the stable association of a multispecies community *Ren Dodge*[1], Eric Jones[7,2], Haolong Zhu[1,3], Benjamin Obadia[5], Chenhui Wang[1], Andrés Aranda-Díaz[4], Kevin Aumiller[1,3], Zhexian Liu[3], Marco Voltolini[8], Eoin Brodie[7], Kerwyn Casey Huang[4,9], Jean Carlson[2], David Sivak[7], Allan Spradling[1,3,6], Will Ludington[1,3] 1) Carnegie Institute of Washington; 2) University of California, Santa Barbara, CA; 3) Department of Biology, Johns Hopkins University; 4) Department of Microbiology and Immunology, Stanford University School of Medicine; 5) Molecular and Cell Biology Department, University of California; 6) Howard Hughes Medical Institute; 7) Simon Frasier University, Burnaby, BC; 8) Lawrence Berkeley National Lab; 9) Chan Zuckerberg Biohub

Animal guts are colonized by a complex community of host-specific commensal bacteria that is relatively stable over time within an individual and can have life-long effects on health. The microbiome is established and maintained in the face of daily fluctuations in diet, invasion by pathogens, and disruptions by antibiotics. Many gut resident bacteria localize to specific regions of the gut that correspond to chemical environments matching the specific species' metabolism. Certain probiotic species, namely Lactobacilli, additionally make physical attachments with host mucus, stabilizing their colonization. A key gap in our knowledge is how the host constructs microenvironments that promote colonization by specific multispecies communities of bacteria. Here, we show that a physical niche exists within the proventriculus region of the Drosophila foregut that selectively binds bacteria with exquisite strain-level specificity. Using gnotobiotic flies, microscopy, and microbial population kinetics we found that primary colonizers saturate the niche and exclude secondary colonizers of the same strain. Conversely, initial colonization by Lactobacillus physically remodels the niche to favor secondary colonization by another commensal, Acetobacter. Our results provide a mechanistic framework for understanding the establishment and stability of a multispecies intestinal microbiome. We anticipate this model will form the basis for dissecting the host genetics of an intestinal niche as well as for the discovery of similar niches in Drosophila and other animals, including humans.

71 The Turandot proteins promote tolerance to stress by regulating energy consumption and tracheogenesis Samuel Rommelaere[1], *Alexia L. Carboni*[1], Jean-Philippe Boquete[1], Shu Kondo[2], Bruno Lemaitre[1] 1) Global Health Institute, School of Life Science, École Polytechnique Fédérale de Lausanne (EPFL), 1015 Lausanne, Switzerland; 2) Invertebrate Genetics Laboratory, Genetic Strains Research Center, National Institute of Genetics, Mishima, Japan

Turandot (*Tot*) genes code for a family of height small secreted proteins. They are highly expressed in response to a variety of stresses. *Tots* are transcriptional targets of several stress pathways, including the JAK/STAT and p38

MAPK pathways. Although *Tots* have been extensively used as readouts of the stress response, they have never been functionally characterized. We have generated a *Drosophila* line that lacks 6 *Tots* (*Tot*^{XMAZ}). These mutant flies are extremely susceptible to several challenges, including bacterial infection, starvation and osmotic stress. Phenotypically, *Tot*^{XMAZ} flies have reduced triglyceride content. During stress exposure, their lipid and carbohydrate stores are rapidly depleted, suggesting that energy availability is a limiting factor in the stress response of these flies. Indeed, feeding *Tot*^{XMAZ} flies a high-sugar diet improves their survival to osmotic stress. *Tots* may thus participate in the stress response by tempering energy utilization. Turandot proteins secreted by the fat body specifically bind to tracheas. Interestingly, tissue tracheation is reduced in *Tot*^{XMAZ} mutant and restoring tracheal growth in these flies is sufficient to rescue their susceptibility to stress. Altogether, our data suggest that Turandot proteins participate in the adaptation to stress by controlling tracheal growth and nutrient utilization. We propose that Turandot may adjust the fly metabolic rate by controlling tracheal homeostasis under stress conditions.

72 Regulation of Misshapen during Border Cell Migration *Gabriela Molinari Roberto*, Gregory Emery Institute for Research in Immunology and Cancer. Université de Montréal

Introduction: Collective cell migration is an important process during development, wound repair and metastasis. Border cells (BC) migration in Drosophila egg chamber is a powerful model to study collective cell migration in vivo. Misshapen (Msn) is a key regulator of this process that coordinates protrusion formation with contractile forces through the border cell cluster. However, the molecular mechanism regulating Msn in border cells is still unknown. Msn is composed of a kinase domain and a Citron homology (CNH) domain separated by a long coiled-coil. The kinase domain was shown to be phosphorylated by the kinase Tao in other contexts, while CNH domains are described to be bind to small GTPases. Therefore, our hypothesis is that both Tao and small GTPases regulate Msn activity during border cell migration.
Methods and Results: Border cell clusters depleted for Tao or Msn are phenotypically identical: they do not entirely detach from the follicle epithelium and they present ectopic protrusions. Interestingly, the expression of a Msn phosphomimetic form restores the migration phenotype induced by Tao depletion. This indicates that the main function of Tao in BCs is to phosphorylate and activate Msn. Furthermore, we found that the CNH domain of Msn is required for its recruitment at the periphery of the cluster, suggesting that its interaction with a GTPase is required for its recruitment. Using the expression of mutant form of Rho and Rap GTPases and co-immunoprecipitation, we found that Rap1 and Rap2l might regulate Msn. In particular, we found that Msn binds preferentially to Rap2L. The role of Rap2L in BC migration is unknown, but preliminary data shows that its depletion induces a migration phenotype. Further analysis of the lost-of-function phenotype and its effects on Msn localization will be performed.
Conclusion and Relevance: Here we presented two mechanisms of regulation of Msn in BC migration. We show that Tao acts as Msn upstream kinase to activate it in BC. Furthermore, we found that Msn binds to Rap GTPases with a preference to Rap2L, which might regulate its recruitment at the periphery of the BC cluster. The Msn ortholog, MAP4K4, is found dysregulated in many cancers and related to metastatic capacity and has been linked to Rap2 and Tao kinases. Therefore, our findings suggest that a conserved molecular cascade regulates the collective cell migration in BCs and during cancer progression.

73 Investigating mechanisms regulating actin assembly in the early *Drosophila* embryo *Anna Yeh*[1], Julian Eskin[2], Bruce Goode[2], Adam Martin[1] 1) MIT; 2) Brandeis University

Actin networks undergo dynamic rearrangements that promote morphogenesis in the developing embryo. Therefore, actin network dynamics must be tightly regulated in space and time. The range of mechanisms that control actin network assembly and organization during development is still not fully understood. The *Drosophila* Synaptotagmin-like protein, Bitesize (Btsz), has been shown to organize actin at epithelial cell apical junctions in a manner that is thought to depend on its interaction with the actin-binding protein, Moesin. Through the maternal depletion of Btsz by RNA interference (RNAi), we discovered that Btsz functions at earlier, syncytial stages of development. In the syncytial embryo, nuclei separated by metaphase furrows undergo rounds of synchronous divisions. Btsz-RNAi disrupts these metaphase furrows, leading to spindle collisions and nuclear division defects, similar to mutants in the formin Diaphanous. Interestingly, we identified an amino acid motif in Btsz, that is similar to a formin elongation effector domain (FEED) found in yeast formin regulators. Additionally, purified Btsz protein containing FEED stimulates formin activity and directly interacts with F-actin through the C-terminal half of the protein *in vitro*. To test the functional requirement of Btsz isoforms that contain this motif, we used CRISPR/Cas9 to engineer a *btsz* mutant, that specifically targets FEED-containing isoforms. Our preliminary data suggest FEED-containing isoforms and to a lesser extent, MBD-containing isoforms are important for the syncytial stages of development. This work suggests a novel mechanism regulating actin assembly during animal development.

74 Discovery of a novel competitive interaction between the *Chlamydia trachomatis* early effector Tarp and the endogenous actin bundler Singed/Fascin during mechanosensory bristle development *George Aranjuez*, Travis Jewett Immunity and Pathogenesis Division, College of Medicine, University of Central Florida, Orlando, FL

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. The early effector Tarp is required for efficient host cell entry by *Chlamydia* since a ΔTarp mutant has significantly reduced invasion frequency. Tarp is a ~150kDa protein with a tyrosine-rich N-terminal region and a C-terminal region that holds actin nucleating as well as F-actin bundling activities. Interestingly, removing Tarp's F-actin bundling domain also impacts *Chlamydia* invasion. Studying the F-actin bundling activity of Tarp within the host cell has not been explored. Here, we use *Drosophila* as an in vivo cell biology platform to study Tarp's F-actin bundling activity and how it impacts host cell biology. Ubiquitous expression of Tarp results in reduced adult viability and tissue-specific expression leads to various defects consistent with its ability to manipulate host actin. We used mechanosensory bristles as a model to study Tarp's F-actin bundling activity since the establishment of bristle shape is dependent on proper F-actin bundling. Expressing Tarp in the thorax of wildtype flies leads to increased curvature of mechanosensory bristles. The growing pupal bristle is supported by parallel longitudinal F-actin bundles and the actin bundler Singed, required for normal bristle shape, localizes with the F-actin bundles. Surprisingly, Tarp expression in the bristles displaced Singed away from F-actin bundles. Tarp's competitive behavior against Fascin during F-actin bundling was confirmed in vitro using co-sedimentation assay. Loss of either *singed* or *forked* in flies leads to highly deformed bristles. Strikingly, Tarp partially rescued the bristle morphology defect caused by *singed* knockdown. This work demonstrates the utility of *Drosophila* in studying bacterial effector function. Moreover, we provide in vivo confirmation of Tarp's F-actin bundling activity and further uncovers a competitive behavior against Singed/Fascin during F-actin bundle assembly.

75 Sufficiency of active Rac to drive whole tissue phagocytosis in vivo *Abhinava Mishra*, Lauren Penfield, Denise Montell University of California Santa Barbara

The Rho family of small GTPases, Rac, Rho, and Cdc42 represent central nodes in the cytoskeletal and signaling networks that drive cell migration and engulfment. How the cells utilize the same RhoGTPase networks to promote different processes in different contexts is not well understood. We address this question in the *Drosophila* ovary, which contains ~850 somatic follicle cells and 16 much larger germline cells. Previously we reported that local and transient activation of Rac using a photoactivatable Rac (PA-Rac) induced protrusions and motility in border cells. However, persistent expression of a constitutively active form of Rac (Rac-CA) in just a subset of follicle cells, using a cell-type-specific enhancer (*slbo-Gal4*), destroyed the entire egg chamber, a phenotype that is significantly suppressed by mutation of a single engulfment receptor, Draper. Additionally, border follicle cells expressing Rac-CA by *slbo-Gal4* engulf adjacent polar cells in early-stage 8 egg chambers. To further assess the effects of Rac-CA mediated engulfment, we generated FlpoutGal4 driven clones to express Rac-CA in a subset of follicle cells. These clones displayed a non-autonomous expression of Draper in follicle cells. Therefore, the expression of Rac-CA in a few cells destroys the whole tissue likely by stimulating engulfment.

To understand the mechanisms underlying Rac-CA mediated tissue destruction, we performed fixed tissue imaging with cell death markers. We observed that expression of Rac-CA caused caspase activation in follicle cells non-autonomously and increased acidification of germline nurse cells evident by lysotracker staining. Live imaging of follicle cells expressing Rac-CA by *slbo-Gal4* unveiled rapid and synchronous death of germline nurse cells. It was initiated by biting the nurse cell membrane by border follicle cells. Expression of Rac-CA by stretch follicle cell-specific *PG150-Gal4* also results in egg chamber death suggesting that the Rac-induced egg chamber death shares some features with late-stage developmental programmed cell death.

These results indicate that focal and transient activation of Rac promotes protrusion and motility, whereas high and sustained Rac activation promotes phagocytosis, initially of living cells, ultimately destroying the whole tissue. These results offer a novel explanation for a previously mysterious human immunodeficiency.

76 WAVE regulatory complex facilitates cell rearrangements through the generation of an F-Actin subpopulation at tri-cellular junction in the follicular epithelium *Lisa Calvary*, Piere Pouchin, Graziella Richard, Vincent Mirouse iGReD, Université Clermont Auvergne - CNRS UMR 6293- INSERM U1103 - Clermont-Ferrand, France

How cells change their respective position to allow a tissue acquiring a specific shape is a major question in development. Drosophila follicle elongates through its antero-posterior axis and this elongation depends on the follicular epithelium. We recently described that this elongation relies on two steps, the first one occurring from stage 3 to 7 and involving, among other mechanisms, cell intercalations (Alégot et al., eLife, 2018). However, in these cells there is no junctional planar polarization of Myosin II as it is the case in some other epithelia undergoing cell intercalations. In this context, we got interested in the role of F-Actin in cell rearrangements.
By performing a reverse genetic screen of F-Actin regulators, we identified the WAVE regulatory complex (WRC), a complex involved in the generation of branched F-Actin, as required for follicle early elongation. In follicular cells, WRC is localized at tri-cellular junctions and colocalized with an intense spot of F-Actin. Tricellular junctions are important hotspots for tissue dynamics thought their function is not yet fully understood. In mutant cells for WRC subunits, this

spot of F-Actin is absent suggesting that it reflects the protrusive activity of this complex. Accordingly, high-resolution analysis reveals that these spots correspond to protrusions from one cell between the bicellular junction of the two others. In addition, mutants cells for WRC exhibit a strong packing defect, leading to the conclusion that WRC is involved in cell rearrangements. We therefore propose that the protrusive activity of WRC at tri-cellular junctions promotes cell intercalations, allowing follicle elongation. Data of our current analyze on living samples of the relationship between F-Actin protrusions induced by WRC and cell junction dynamics will be presented.

77 Fat2 polarizes the WAVE complex to align protrusions for collective cell migration *Audrey Williams*, Seth Donoughe, Edwin Munro, Sally Horne-Badovinac University of Chicago

Many epithelial tissues form tubes and spheres, rather than the sheets often studied using epithelial cell culture models. Despite their closed topology, examples of spherical or tubular tissues undergoing collective cell migrations have emerged. The follicle cells of the ellipsoidal Drosophila egg chamber undergo one such collective migration. These epithelial cells, which make up the outermost cell layer of the egg chamber, migrate along an encapsulating basement membrane with high directional persistence for days. In the absence of a free edge or another guidance cue to dictate their migration direction, they must coordinate their migration through local interactions within the migrating cohort. One important regulator of follicle cell migration is the atypical cadherin Fat2, which localizes to the trailing edge of each cell and promotes leading edge protrusion of the cell behind. Another is the WAVE regulatory complex, which templates the formation of lamellipodial protrusions at each cell's leading edge. Using genetic manipulations, live imaging, and quantitative image analysis, we show that Fat2 polarizes protrusive activity *in trans* by concentrating the WAVE complex at the leading edge, across the cell-cell interface. Without this cue from Fat2, the WAVE complex remains active and the cells are protrusive, but protrusive sites fluctuate around the cell periphery and collective cell migration fails. To stabilize the orientation of WAVE complex enrichment and protrusive activity, clusters of Fat2 recruit the WAVE complex to corresponding clusters just across the cell-cell interface, at the tips of filopodia embedded within the lamellipodium. Because the Fat2-WAVE complex signaling system is deployed at each leading-trailing interface in a planar polarized manner, it both polarizes protrusions within individual cells and couples these individual cell polarities across the epithelium. This allows the cells to exert force in a common direction and achieve a highly coordinated collective cell migration.

78 Defining the role of prostaglandins in collective cell migration *Samuel Mellentine*, Anna Ramsey, Omar Rabab'h, Tina Tootle University of Iowa Carver College of Medicine

Collective cell migration – the coordinated movement of associated cells – is important for both normal development and tumor invasion. Prostaglandins (PGs) are short-range lipid signals that regulate cell migration and are up regulated in many cancers. However, their mechanisms of action during collective migration are poorly understood. Here we use the native, 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) in border cell migration. Our data support the model that cPGES and PGFS are required for on-time border cell migration. Specifically, loss of cPGES or PGFS delays border cell migration, but has no effect on cluster cohesion. 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 to promote border cell migration. We are also assessing genetic interaction between the synthases and Pxt. We find that co-reduction of both Pxt and PGFS results in delayed border cell migration; supporting that the phenotype due to loss of PGFS is related to loss of PG signaling. Together our data lead to the model that PGE_2 and $PGF_{2\alpha}$ both promote on-time border cell migration. As PG signaling is highly conserved, these studies provide critical insight into the specific functions of individual PG signaling cascades controlling collective cell migration and can be applied to understanding both developmental collective cell migrations and pathological migrations including cancer metastasis.

79 Microtubule acetylation promotes epithelial cell stretching and squamous cell carcinogenesis in *Drosophila* *Rachita Bhattacharya*, Nitin Mohan, Pradip Sinha Indian Institute of Technology Kanpur, India

The cytoskeleton is an essential regulator of cell shape and size changes, of which microtubules (MT) are a crucial component. Indeed, MT re-organize during epithelial cell shape transition, for instance, from columnar to the squamous cell shape. The mechanism that regulates these MT-mediated cell shape remodeling, however, is not known. Here, we show that MT acetylation is an essential step during epithelial cell stretching. For instance, MT acetylation increases remarkably during columnar-to-squamous transition. Conversely, loss of MT acetylation via genetic downregulation of Tubulin Acetyl Transferase (TAT) compromises the cell stretching by dysregulating the turnover of polarity protein, Crumbs, and cell-cell adhesion protein, cadherin. We further show that neoplastic transformation of squamous epithelium is accompanied by hyperacetylation of MT. Our results, therefore, reveal an essential mechanism of MT-

acetylation-dependent cell shape regulation as well as squamous cell carcinogenesis and hints at potential therapeutic targets.

80 Maternal pioneer factor CLAMP regulates sex-specific transcript diversity in early *Drosophila* embryos. *Mukulika Ray*[1], Ashley Conard[2], Jennifer Urban[3], Annie Huang[1], Erica Larschan[1] 1) MCB department, Brown University, Providence, RI; 2) CCMB department, Brown University, Providence, RI; 3) Johns Hopkins University, Baltimore, Maryland

Post-transcriptional processes of alternative splicing of RNA are essential in gene expression, critical for transcript diversity, eventually leading to cellular differentiation and specification. Alternative splicing is initiated during the initial embryonic stages when the key gene expression regulators are maternally deposited transcription factors (TFs). Yet, it is unknown how transcription factors (TFs) work with splicing factors to regulate alternative splicing. Also, how maternal factors shape the early embryonic transcriptome in a sex-specific manner is not well understood. Here, we show that the maternally deposited transcription factor CLAMP (Chromatin-linked adaptor for MSL proteins) is essential for sex-specific alternative splicing in early embryos of both sexes. We found that CLAMP binds along with the gene bodies of sex-specifically spliced genes in both sexes and differentially binds to RNA-binding protein components of the spliceosome complex in males and females. In females, CLAMP also modulates the chromatin environment at the *sxl* gene, the master regulator of sex-determination, affecting its splicing and thus downstream female-specific splicing events. In males, CLAMP influences the distribution of RNA-helicase Maleless (a component of spliceosome complex and Male Sex-lethal (MSL) complex in males) on chromatin, affecting male sex-specific splicing. We show that CLAMP is an RNA binding protein through iCLIP (individual-nucleotide resolution Cross-Linking and Immunoprecipitation) and RNA gel shift experiments and is important for the dynamics of formation of nuclear speckles of ribonucleoprotein origin that constitute alternative splicing complexes. Using CLAMP mutants, we demonstrate that the prion-like domain (PrLD) of CLAMP inhibits aggregation of RNA-binding splicing factors that renders them non-functional and is essential for survival. We hypothesize that the PrLD domain alters the phase transition properties of CLAMP. Thus, we define a new function for a maternally deposited TF in regulating sex-specific alternative splicing via interaction with chromatin and RNA-binding proteins, which influences the distribution of RNA-binding proteins on chromatin and between protein complexes. As a part of this study, we have also developed a computational pipeline called time2splice for the identification of alternative splicing events which change over time and between sexes.

81 Hippo pathway transcriptional regulators alter chromatin binding dynamics of the transcription factor Scalloped *Samuel Manning*[1,2], Benjamin Kroeger[1,2], Elizabeth Hinde[3], Kieran Harvey[1,2] 1) Monash University, Melbourne, Australia; 2) Peter MacCallum Cancer Centre, Melbourne, Australia; 3) The University of Melbourne, Australia

The Hippo signalling pathway is a highly conserved regulator of cell fate and organ size, which regulates the nuclear-cytoplasmic shuttling of the transcriptional co-activator Yorkie (Yki), and thereby regulates gene expression. We currently have very little understanding of how the Hippo pathway modifies the nuclear biophysical behaviour and genomic interactions of Yki or its DNA binding partner Scalloped (Sd) to regulate transcription.
To address this, we used cutting-edge ex vivo fluorescence microscopy approaches including Raster Image Correlation Spectroscopy (RICS), Fluorescence Recovery After Photobleaching (FRAP) and Single Molecule Tracking (SMT) to investigate the biophysical behaviour of Sd and Yki in the nuclei of living Drosophila tissues. We found that Yki moves more rapidly through the nuclear environment than Sd, and that Yki's movement is highly sensitive to Sd abundance, demonstrating the importance of Sd for Yki's navigation of the genome. Sd and Yki were highly enriched at regions of active chromatin, consistent with their role as a heterodimeric activator of transcription. Further, enrichment of Sd at these loci is driven in part by increased chromatin residence times. Yki interacts more transiently with chromatin than Sd at these sites, and the duration of this interaction is dependent on Sd levels, further highlighting the importance of Sd levels for recruitment of Yki. FRAP and SMT demonstrate that Yki and the transcriptional repressor Nerfin-1 alter Sd chromatin residence times, providing mechanistic insight in to how these proteins alter Hippo pathway output.
Taken together we show that a transcriptional co-activator and repressor are able to modify the binding duration of a transcription factor, with subsequent impacts on transcription. These results also indicate that altering binding duration is one mechanism by which Hippo pathway transcription factors can be enriched at a specific locus to regulate gene expression. These results have implications for understanding how the Hippo pathway regulates normal development and disease, and also provide mechanistic frameworks for understanding how transcription may be regulated by other signalling pathways.

82 Hox linker domain phosphorylation alters Exd-Hox DNA-binding preferences and regulates gene expression *William Glassford*, Richard Mann Zuckerman Mind Brain Behavior Institute, Columbia University, New York, NY

Hox proteins are transcription factors best known for patterning segment identity along the anterior-posterior axis in early embryogenesis. As monomers, Hox proteins exhibit little difference in DNA-binding specificity, however; upon binding their common cofactor Extradenticle (Exd) each Hox paralog gains the DNA-binding specificity necessary to enact

segment-specific gene regulation. Hox-Exd binding is facilitated by a conserved motif in Hox that, when bound to an Exd binding pocket, positions a variable "linker" domain in close proximity to the DNA minor groove. The composition of each Hox's linker domain contributes to paralog-specific Hox-Exd DNA-binding preferences. Recently published work and our own data have found that several Hox proteins can be phosphorylated in their linker domain, leading us to hypothesize that phosphorylation in this domain could influence Hox-Exd DNA-binding preferences. To test this hypothesis, we used genome expansion technology to express and purify Hox proteins with site-specific phosphorylation, and performed the *in vitro* DNA-binding assay SELEX-seq on phosphorylated and non-phosphorylated Hox-Exd complexes. We observed that for two Hox proteins, phosphorylation induced differences in DNA-binding preferences. Interestingly, phosphorylation of one of these Hox's promoted sequences that were reduced by phosphorylation for the other, indicating that phosphorylation may act as a mechanism to further differentiate Hox paralogs. To investigate the *in vivo* function of Hox linker domain phosphorylation, we used genome-engineering to introduce an alanine substitution at one of the predicted Hox phosphorylation sites. Using asymmetric morphogenesis as a model Hox-regulated gene network, we identified one enhancer that, in a transgenic reporter system, exhibited increased expression in a phospho-mutant genetic background. Within that enhancer we identified one conserved Hox-Exd binding site predicted to be more strongly bound by phosphorylated Hox-Exd by our SELEX-seq data. Mutating this site in the context of our transgenic reporter also induced an increase in expression, suggesting that phosphorylation of the linker domain can directly alter gene expression through a phospho-sensitive binding site. These data highlight the capability of post-translational modifications to alter Hox-Exd DNA-binding preferences and that these changes are significant enough to affect the transcriptional activity of Hox-regulated enhancers.

83 Assembly of the Brain tumor RNA decay pre-complex expedites downregulation of Notch signaling following asymmetric stem cell division *Hideyuki Komori*[1], John Bugay[1], Hua Luo[2], Craig Smibert[2,3], Howard Lipshitz[2], Cheng-Yu Lee[1] 1) Life Sciences Institute, University of Michigan, Ann Arbor, MI, USA; 2) Department of Molecular Genetics, University of Toronto, Toronto, ON, Canada; 3) Department of Biochemistry, University of Toronto, Toronto, ON, Canada

RNA-binding proteins assemble translational control complexes by recruiting their interactors that exert regulatory effects on target mRNAs. How these multi-protein complexes are assembled in a timely manner to promote dynamic changes in gene regulatory programs that promote proper specification of cell identity during key developmental transitions remains poorly understood. During asymmetric division of type II neuroblasts in fly larval brains, RNA-binding protein Brain tumor (BRAT) segregates into one of the neuroblast progeny in which it expedites the decay of NOTCH target gene transcripts allowing for the onset of differentiation less than 60 minutes after division. The tight correlation between BRAT-mediated mRNA decay and differentiation initiation following asymmetric neuroblast division provides an excellent *in vivo* paradigm for testing temporospatial control of the assembly of BRAT-containing RNA regulatory complex. We confirmed that NOT1 and PAN3, the scaffolding components of two main RNA deadenylation complexes, co-segregate with BRAT in mitotic neuroblasts and that their segregation is BRAT-dependent, consistent with our previous findings that Brat functions together with these proteins to promote the decay of NOTCH target gene transcripts. We also identified Ubiquitin specific protein 5 (USP5) as a novel BRAT interactor by IP-MS experiment using embryonic extract. We confirmed that USP5 co-segregates with BRAT into neuroblast progeny destined to differentiate, and that asymmetric USP5 segregation depends on BRAT. Furthermore, reducing *usp5* function enhanced differentiation defects in *brat*-hypomorphic brains, and complete loss of *usp5* leads to supernumerary neuroblast formation phenocopying *brat* mutant. By using single-molecule fluorescent RNA *in situ* hybridization, we demonstrated that neuroblast progeny ectopically accumulate Notch target gene transcripts in *brat* or *usp5*-mutant brains, supporting the model that USP5 is a key component of the BRAT RNA decay complex. The scaffolding protein Miranda (MIRA) binds and segregates BRAT in mitotic neuroblasts. Consistent with Miranda inhibiting the RNA-binding ability of BRAT in wild-type neuroblasts, we detected co-localization of NOTCH target mRNAs with mutant BRAT that is defective in MIRA-binding in mitotic neuroblasts. We propose that BRAT assembles an enzymatically inactive and non-RNA-binding pre-complex in mitotic neuroblasts, and the BRAT complex becomes activated in neuroblast progeny by recruiting the enzymatic components of the RNA deadenylation complexes and dissociation from MIRA.

84 Using CRISPRi to uncover mechanisms of transcriptional repression by Rb and CtBP co-repressors *Ana-Maria Raicu*[1], Patricia Castanheira [2], David Arnosti[2] 1) Cell and Molecular Biology Program, Michigan State University, East Lansing, MI; 2) Department of Biochemistry and Molecular Biology, Michigan State University, East Lansing, MI

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) and the C-terminal binding protein (CtBP), which are highly conserved co-repressors across Metazoa. In *D. melanogaster*, the Rb family consists of the Rbf1 and Rbf2 paralogs, while a single CtBP gene encodes the CtBP-long and CtBP-short isoforms, which differ by about 100

residues in the CTD. We expressed the dCas9-Rb and dCas9-CtBP chimeras in the fly wing for *in vivo* targeting to specific promoters, and tested each protein's effect on target gene expression and their mechanism of repression. We found that on some genes, both Rbf1 and Rbf2 are able to mediate potent repression, but that this happens in a distance-dependent manner. In contrast, we found examples where Rb proteins modestly activate the target gene, suggesting that they do not always function to turn off transcription. Notably, an Rbf1 CTD mutant lacking the Instability Element (IE), which we previously identified as being necessary for activity, was just as good a repressor as the wild type protein. This suggests that the IE is chiefly involved in recruiting and is not necessary for repression. Between the CtBP isoforms, CtBP-short was a much more potent repressor than CtBP-long, which indicates that the long CTD extension may have an inhibitory role in transcription. 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 and CtBP proteins will enhance our understanding of these conserved regulatory proteins in development and disease.

85 Sculpture of a sex-specific piRNA program *Peiwei Chen*, Alexei Aravin California Institute of Technology

Maternally deposited piRNAs act as epigenetic vectors to pass on transgenerational memory of selfish genetic elements to the offspring. This model posits that the maternal input instructs the zygotic genome to mount a piRNA program in the next generation that reflects the maternal response to genomic parasites. However, males implement a piRNA program distinct from the maternal instruction and their female siblings. Here, we disentangled the contribution of three factors – sex chromosome content, gonadal sex, and maternal input – towards a sex-specific piRNA program. While maternally deposited piRNAs can identify homologous sequences as piRNA-producing loci in the zygote, we found *de novo* piRNA production that does not rely on maternal input but instead depends on the chromosome content and gonadal sex. In fact, Y chromosome is both necessary and sufficient to recapitulate some key aspects of male piRNA program. Meanwhile, sexual identity exerts a major influence on divergent piRNA production from identical genomic sequences between sexes. Our work showed that it is the collective action of chromosome content, gonadal sex and maternal input that sculpts the observed sexual dimorphism in piRNA program, highlighting a previously unknown input from sexual identity into piRNA biogenesis.

86 The steroid hormone Ecdysone coordinates larval growth and development through its interaction with the transcriptional repressor Smrter. *Joanna Wardwell-Ozgo*, Colby Schweibenz, Douglas Terry, Ken Moberg Emory University School of Medicine

Hormones act as systemic signaling cues to coordinate developmental timing, growth, and tissue patterning. In *Drosophila,* pulses of the steroid hormone ecdysone (Ec) synchronize various physiological and developmental processes required for larval ecdysis, tissue growth, and morphogenesis. Binding of the bioactive form of ecdysone (20-hydroxyecdysone; 20E) to its cognate nuclear hormone receptor, Ecdysone Receptor (EcR), is proposed to switch target genes from a transcriptionally repressed to activated state. Current data indicate that EcR has tissue-specific targets, and that even within a single cell type, developmental fluctuations in 20E levels also coincide with dynamic redistribution of EcR between different loci. Despite a long history of genetic analysis of EcR, mechanisms that direct these temporal/cell-type specific transcriptional responses to 20E are not well defined. To probe this repression-to-activation switch in a single tissue, we created a Gal4/UAS-regulated "EcR sponge" transgene composed of the ligand binding domain (LBD) region of EcR (EcR^{LBD}) fused to RFP. Expression of EcR^{LBD} represses heterologous EcR transcriptional reporters in wing disc cells when 20E titers are high (late L3), and reciprocally activates EcR activity in these same cells during early L3 when 20E levels are low, indicating that EcR^{LBD} interacts with key elements of the repression-to-activation switch. Indeed, loss of the transcriptional repressor Smrter (NCoR) mimics the derepressing effect of EcR^{LBD} in early L3 wing cells, suggesting that Smrter is an EcR-associated repressor in larval disc cells. Consistent with this hypothesis, a version of EcR^{LBD} carrying the A483T mutation, which blocks Smrter binding, (termed 'EcR^{Dmber}') represses EcR transcriptional reporter activity regardless of developmental age. With regard to endogenous target genes, EcR^{LBD}, but not EcR^{Dmber}, relieves repression of multiple EcR targets in wing disc cells and disrupts some, but not all, 20E-EcR-triggered developmental processes in the larval salivary gland and fat body. Significantly, expression of EcR^{LBD} confers a growth advantage to larval wing cells and derepresses a subset of growth genes controlled by the Hippo pathway. In sum, these data reveal distinct molecular roles of 20E-EcR signaling at individual promoters, and suggest that 20E-EcR signaling coordinates proliferation and tissue growth in part through repression of Hippo pathway target genes. Current work centers on proteomic analysis of EcR^{LBD}interactors in larval cells to identify key activators and repressors that are active in these mechanisms.

87 Embryo development requires histone acetylation by Nejire during the maternal-to-zygotic transition *Audrey Marsh*[1], George Hunt[2], Tyler Gibson[1], Elizabeth Larson[1], Katherine Hullin[1], Mattias Mannervik [2], Melissa Harrison[1] 1) Department of Biomolecular Chemistry, University of Wisconsin School of Medicine and Public Health, Madison, WI; 2) Department of Molecular Biosciences, The Wenner-Gren Institute, Stockholm University, 10691, Stockholm, Sweden

The sperm and egg are differentiated cell types, individually specialized for the purpose of fertilization. After merging

to form the diploid genome, the new embryo must remove signatures in the parental genomes to reprogram to a totipotent state. Reprogramming is essential for the embryo to give rise to all the cell types of the adult organism. This reprogramming process occurs during a period called the maternal-to-zygotic transition (MZT). Initially transcriptionally quiescent, the zygotic genome is gradually 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 their unique 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, bound to thousands of genomic loci that are also occupied by ZLD and that expression of ZLD in tissue culture resulted in increased acetylation at ZLD-bound loci. In the early embryo, ZLD knock down resulted in decreased acetylation and NEJ recruitment at shared sites. Together these data suggest ZLD mediates activation of the zygotic genome, at least in part, by recruiting NEJ to acetylate histones. To test whether NEJ-mediated acetylation during the MZT is required for development, we used the CRY2 optogenetic system to specifically inactivate NEJ during the period of widespread zygotic genome activation. Embryos treated with blue light through the MZT failed to hatch as compared to sibling controls left in the dark, indicating that NEJ is necessary during this early time in development. Our findings suggest a model in which pioneer factors are first to prime the genome for activation by recruiting histone acetyltransferases to *cis-regulatory* regions and that this is required for genome activation. Ongoing work is investigating the role of NEJ in activating the zygotic genome and establishing the mechanistic relationship between NEJ and the pioneer factor ZLD.

88 Color augments motion vision for detecting approaching objects in *Drosophila* *Kit D Longden*, Edward M Rogers, Aljoscha Nern, Heather Dionne, Michael B Reiser HHMI Janelia Research Campus

Color and motion are used by many species to identify salient moving objects. They are processed largely independently, but color contributes to motion processing in humans, for example, enabling moving colored objects to be detected when their luminance matches the background. In *Drosophila*, motion vision was thought to be independent of color vision, based on experiments with blue and green gratings; color inputs to the luminance system had been identified through neurogenetics and connectomics, but these were thought to expand the wavelength sensitivity of the luminance channel. Here, we demonstrate an unexpected, additional contribution of color to motion vision in *Drosophila*, using a novel display system to present ultraviolet (UV) and green visual patterns to tethered flying flies. We show that *Drosophila* respond to looming UV discs at all intensities, indicating that color contributes to their motion vision. We further show that behavioral ON-motion responses are more sensitive to UV than for OFF-motion, and this difference is present in all six *Drosophila* species we tested. Using genetic rescue and silencing, we show that ON-motion UV-sensitivity depends on the UV-sensitive R7 photoreceptors, and using calcium imaging of neural activity we show that ON-motion encoding T4 cells are more sensitive to UV than OFF-motion encoding T5 cells. Calcium imaging of the cells connecting the photoreceptors to T4 reveals a diversity of UV-sensitivity, in which the UV-sensitivity of medulla T4 input cells correlates with that of their most numerous lamina monopolar cell inputs, notably Mi9 and L3. In a parallel connectomics study, we discovered that the majority of R7 inputs to L3 are found in the optic chiasm between the medulla and lamina, and so had been missed in previous work. We hypothesized that if the ability of flies to respond to all intensities of looming UV discs was explained by ON-motion sensitivity to UV, then flies should be motion blind to looming green discs with a UV background, even when they have the same chromatic contrast as UV discs that drive behavioral responses. Remarkably, behavioral experiments confirmed this hypothesis. Together, these results demonstrate that color contributes to motion vision in *Drosophila* in a way that favors the detection of approaching UV objects. We propose that UV objects are of particular interest to flies, and illustrate how this mechanism generalizes for the detection of approaching colored objects in other visual systems.

89 A hymenopteran odorant alerts flies to bury eggs *Shaun Davis*[1], Gregory Chism[1], Megan Maurer[2], Julio Trejo[1], Ricardo Garcia[1], Todd Schlenke[1] 1) University of Arizona, Tucson, AZ; 2) Arizona State University, Tempe, AZ

Ants are ubiquitous and consume insects at all life stages, presumably creating a strong selective pressure for ant avoidance behaviors across insects. The insect egg stage can be especially defenseless against predation given that eggs are usually immobile and unguarded, suggesting insect mothers may have evolved oviposition strategies to minimize the ant predation risk to their offspring. Given the lack of parental care in most insects, these oviposition strategies would likely be innate rather than learned, since insect mothers are not usually present to assess predation of their eggs. Here, we use the vinegar fly *Drosophila melanogaster* as a model system for examining parental defensive responses to ant presence. Flies usually lay eggs partially inserted into the food substrate, although some are laid on top of the food and a few are inserted deeply into the food. We found that exposure to ants significantly alters fly oviposition depth: the proportion of eggs on the food surface decreased while the proportion of buried eggs increased. Buried eggs survive ant foraging bouts better than surface eggs, showing that this oviposition depth behavior is adaptive. This induced behavior is conserved across the genus Drosophila and is dependent on the fly olfactory system: anosmic mutant flies fail to bury

their eggs in the presence of ants, and ant odor extracts are sufficient to induce egg burying. By fractionating ant body washes and using GC-MS to identify fraction constituents, we identified the saturated, long-chain alcohol 1-octadecanol as the odorant flies use to sense ant presence. To further delineate the ant lineages to which flies respond, we exposed flies to the odors from numerous species of ants and other insects. Surprisingly, flies buried their eggs in response to the odors of nearly all hymenopterans tested, including hymenopteran groups that flies rarely interact with in nature like bees and paper wasps. Our data suggest that 1-octadecanol is a conserved and ancient hymenopteran odorant, and that drosophilids evolved a mechanism for sensing this odorant early in their evolution as a means of protecting their offspring from ant predation. This study sheds light on the ecology and mechanisms underlying a common biotic interaction in nature, that between insect parents and the ants that would consume their offspring.

90 STIM dependent dopamine-neuropeptide axis maintains the larval drive to feed and grow. *Nandashree Kasturacharya*[1,2], Gaiti Hasan[1] 1) National Centre for Biological Science; 2) The University of Trans-Disciplinary Health Sciences and Technology (TDU)

Feeding is a vital and complex behavior essential to acquire nutrition. In Drosophila melanogaster, a holometabolous insect, the extent and nutritional quality of food ingested as larvae determines the adult size and ultimately fecundity. Studies on Drosophila larval feeding behavior have shown that under different nutritional conditions, multiple neuronal circuits regulate and maintain feeding. But most of these studies focused on the later stage of larval feeding and less is known on how constant feeding is maintained in second instar larvae. Here we have identified a subset of dopaminergic (THD') neurons that maintain constant feeding in early larvae. The function of STIM protein (an ER calcium sensor) in this subset of dopaminergic neurons is important to maintain their excitability and dopamine release. Increasing the activity in THD'-dopaminergic neurons through genetic means rescued the feeding deficit of $STIM^{KO}$ larvae. Through optogenetic methods, we have established that THD'-dopaminergic neurons are synaptically connected to a subset of neuropeptidergic cells. Although dopaminergic neurons are known to regulate feeding behavior under different conditions, this is the first study showing how a subset of dopaminergic neurons regulate larval growth and pupariation by maintaining constant feeding.

91 A taste for toxins: Evolution of feeding preferences in the herbivorous drosophilid Scaptomyza flava *Julianne Pelaez*, Noah Whiteman University of California, Berkeley

Herbivorous insects account for half of all insect species—their evolutionary success is hypothesized to have been driven by co-diversification with their host plants over the last 400 million years. During this dynamic dietary shift, herbivores need to evolve detoxification mechanisms to overcome toxic plant secondary compounds, but also behavioral changes to preferentially feed and lay eggs on their new host. While highly specialized herbivores use these toxins or their precursor molecules to identify their hosts, in the early stages of herbivore evolution, the taste system must first lose or reduce their ancestral aversion to these toxins, while maintaining discriminatory capabilities to distinguish varying levels or different chemical classes of the toxin. The latter may be advantageous for evolutionarily young herbivores lineages that lack sophisticated detoxification systems. How these gustatory adaptations are achieved at the molecular and neural level remains unclear. The herbivorous drosophilid Scaptomyza flava belongs to a lineage that has recently evolved herbivory (~15 million years). This species feeds on mustard plants (Brassicaceae), whose main defense compounds are isothiocyanates, highly reactive electrophiles, derived from glucosinolates, which are used by many mustard specialists as feeding and oviposition cues. Using a dye-based feeding assay, we demonstrate that S. flava has lost aversion to several glucosinolates, compared to closely related non-herbivores (S. pallida, S. shui, and Drosophila melanogaster), while maintaining aversion to the aliphatic glucosinolate sinigrin and other non-mustard plant toxins (caffeine and lobeline). Comparative genomic analyses across herbivorous (3) and non-herbivorous (6) drosophilids, indicate that the herbivorous lineage experienced significant gene losses, gains, and changes in selective constraint among several gene families involved in gustatory reception in the peripheral nervous system. Notably, many of these changes were among gustatory receptors whose homologs in D. melanogaster are expressed in all, or most, bitter sensitive sensilla. Further investigations into the functional role of these genetic changes will allow us to identify how aversion to some toxins is maintained, while lost for others.

92 The mRNA-binding protein Pumilio pleiotropically regulates food-related phenotypes through *foraging* *Ina Anreiter*[1], Zixuan Xiao[1], Vanessa Montemurri [2], Oscar Vasquez[1], Aaron Allen[1,3], Craig Smibert[1], Jeffrey Dason[1,2], Marla Sokolowski[1] 1) University of Toronto; 2) University of Windsor; 3) Oxford University

The regulation of two or more phenotypes by a single gene, behavioural pleiotropy, is a common occurrence. At the molecular level, these genes need to have structural and/or regulatory features that allow for this pleiotropy to occur. Here we dissect the regulation of independent *Drosophila* larval phenotypes through gene product specific regulation of the *foraging* gene by the 3'UTR binding protein Pumilio. *foraging* regulates larval movement on food (pathlength), food-intake, fat stores and nociception. We found that Pumilio binds the 3'UTR of *foraging* mRNA transcripts, regulating expression of the gene in a transcript and protein isoform-specific manner. While overall protein and mRNA levels are upregulated in Pumilio hypomorph mutants, specific transcripts are downregulated. We find that this transcriptional

regulation of *foraging* by Pumilio plays a role in regulation larval food-intake, fat stores and nociception, but not larval pathlength. Interestingly, we find that in the case of larval fat stores and nociception, reduced Pumilio phenocopies the effect of a *foraging* over-expression phenotype, while in the case of larval food-intake reduced Pumilio phenocopies the effect of a *foraging* null phenotype. Together these findings suggest that Pumilio is a transcriptional regulator involved in the molecular regulation of pleiotropy by independent regulation of distinct products of a single gene, *foraging*.

93 Genetic Variation in Cocaine Preference in the *Drosophila melanogaster* Genetic Reference Panel *Jeffrey Hatfield*, Trudy Mackay, Robert Anholt Clemson University, Center for Human Genetics, Greenwood, SC

Studies on the genetic basis of susceptibility to cocaine addiction in human populations are quite challenging due to limited sample sizes, heterogeneity of genetic backgrounds, and environmental variability. *Drosophila* present a powerful model for investigation into the genetic underpinnings of cocaine addiction, using preference for cocaine as a proxy for addiction behavior. Utilizing the Microplate Feeder Assay (MFA), I quantified cocaine preference for 16,442 flies across 103 distinct genetic backgrounds of the *Drosophila melanogaster* Genetic Reference Panel (DGRP). I provided individual flies with an equal choice between 10µL of a control liquid food (4% sucrose, 1% yeast extract, and 0.004% FD&C Blue #1) and 10µL of the same liquid food supplemented with 0.02% cocaine. Solutions were delivered with the MFA, and consumption of each solution was quantified using a plate reader following a 22-hour exposure. Normalized cocaine preference was calculated for each fly as the difference in consumption between the two solutions divided by their total consumption. I found significant, naturally-occurring genetic variation for cocaine preference across this subset of the DGRP. I also observed significant sexual dimorphism across tested DGRP lines, where male flies on average exhibit higher cocaine preference than female flies of the same line. Overall, males of 18.4% of tested DGRP lines preferred cocaine-supplemented food over control food, while females of only 3.9% of lines preferred the cocaine solution. Estimates of broad sense heritability of consumption were calculated using individual level data, as well as using DGRP line means, and were found to be $H^2 = 0.19$ and $H^2 = 0.97$, respectively. Here, we demonstrate the impact of genetics on susceptibility to cocaine addiction behavior in *D. melanogaster*. These data will facilitate future genome-wide association analyses, as well as identify *D. melanogaster* genetic backgrounds from the DGRP that can better model cocaine addiction.

94 Descending neurons coordinate anterior grooming behavior in *Drosophila* *LI GUO*, Neil Zhang, Julie Simspon University of California, Santa Barbara

The brain coordinates the movements that constitute behavior, but how descending neurons (DNs) convey the myriad of commands required to activate the motor neurons of the limbs in the right order and combinations to produce those movements is not well understood. Here, we used fly anterior grooming to address this question. Anterior grooming consists of two basic movements, head sweeps and front leg rubbing. The analysis of the anterior grooming structure by Automatic behavior Recognition System (ABRS) showed that head sweeps and front leg rubbing are normally coupled, but alternate for the efficient dust removal. Using the optogenetic activation, we identified 3 different groups of command-like DNs that can induce front leg rubbing (DNg11), or head sweeps (aDN), or the alternation of both (DNg12). This demonstrates that there are multiple control modes for anterior grooming at the level of descending neurons, which DNs can evoke either the whole alternation program or the specific subroutines. Head sweeps and front leg rubbing both use the front legs and so are mutual exclusive. Parallel optogenetic activation of DNg11 and aDN resulted in normal alternation between head sweeps and front leg rubbing, and DNg12 itself can induce both movements. These results demonstrate that the VNC can resolve conflicting descending drives. Using FlyWire to reconstruct neurons in a whole-brain electron microscope dataset (EM), we identified a novel inhibitory circuit connecting head sensory neurons to DNg11 that command front leg rubbing. This suggests that decisions between actions can also be made in the brain. Lastly, we explored descending control of limb coordination in these movements by unilateral activation of DNs. Interestingly, unilateral activation of DNg12 or aDN1 induced single-side head sweeps, while unilateral activation of DNg11 induced bilateral front leg rubbing. Our results suggests that left and right leg movements can be decoupled in head sweeps but not in front leg rubbing. Using EM, we found a neural circuit in the brain indirectly connecting the left and right DNg11, which can potentially explain how unilateral activation can result in bilateral execution. Taken together, these results demonstrate that distinct descending neurons can orchestrate the complex alternation between the movements that make up anterior grooming.

95 Drosophila females receive male substrate-borne signals through specific leg neurons during courtship *Caroline Fabre*, Eleanor McKelvey University of Cambridge

Substrate-borne vibratory signals are thought to be one of the most ancient and taxonomically widespread communication signals among animal species, including Drosophila flies. During courtship, the male Drosophila abdomen tremulates to generate vibrations in the courting substrate.These vibrations coincide with nearby females becoming immobile, a behavior that facilitates mounting and copulation. It was unknown how the Drosophila female detects these substrate-borne vibratory signals. We present data showing that the immobility response of the female to the tremulations is not dependent on any air-borne cue. We show that substrate-borne communication is used by wild Drosophila and that the vibrations propagate through those natural substrates (e.g., fruits) where flies feed and court.

We examine transmission of the signals through a variety of substrates and describe how each of these substrates modifies the vibratory signal during propagation and affects the female response. Moreover, we identify the main sensory structures and neurons that receive the vibrations in the female legs, as well as the mechanically gated ion channels Nanchung and Piezo (but not Trpγ) that mediate sensitivity to the vibrations. Together, our results show that Drosophila flies, like many other arthropods, use substrate-borne communication as a natural means of communication, strengthening the idea that this mode of signal transfer is heavily used and reliable in the wild. Our findings also reveal the cellular and molecular mechanisms underlying the vibration-sensing modality necessary for this communication.

96 *chinmo*-mutant spermatogonial stem cells cause mitotic drive by evicting non-mutant neighbors from the niche *CHEN YUAN TSENG*[1], Michael Burel[1], Michael Cammer[1], Sneh Harsh[1], Maria Sol Flaherty[1], Stefan Baumgartner[2], Erika Bach[1] 1) NYU Grossman School of Medicine, New York, NY; 2) Department of Experimental Medical Sciences, Lund University, 22184 Lund, Sweden

Niches maintain a finite pool of stem cells via restricted space and short-range signals. Stem cells compete for limited niche resources, but the mechanisms regulating competition are poorly understood. Using the *Drosophila* testis model, we show that germline stem cells (GSCs) lacking the transcription factor Chinmo gain a competitive advantage for niche access. Surprisingly, *chinmo*$^{-/-}$ GSCs rely on a new mechanism of competition in which they secrete the extracellular matrix protein Perlecan to selectively evict non-mutant GSCs and then upregulate Perlecan-binding proteins to remain in the altered niche. Over time, the GSC pool can be entirely replaced with *chinmo*$^{-/-}$ cells. As a consequence, the mutant *chinmo* allele acts as a gene drive element: the majority of offspring inherit the allele despite the heterozygous genotype of the parent. Our results suggest the influence of GSC competition may extend beyond individual stem cell niche dynamics to population-level allelic drift and evolution.

97 Role for local ecdysone signaling in *Drosophila* imaginal wing disc regeneration *Douglas Terry*, Can Zhang, Joanna Wardwell-Ozgo, Colby Schweibenz, Ken Moberg Emory University

Ecdysone steroid hormone (Ec) signaling is required to drive *Drosophila* developmental transitions and to promote growth of larval imaginal discs. Previous studies investigating imaginal wing disc regeneration have demonstrated the importance of inhibiting systemic Ec production to delay development and allow time for regeneration to occur. However, local roles for Ec signaling within regenerating tissues are not well understood. Based on our observation that Ec-responsive transcriptional reporters can be induced locally in injured larval wing discs, we have tested elements of the Ec pathway for roles in regeneration. We find that local knockdown of the Ec biosynthetic enzyme *phantom* (*phm*) during injury is sufficient to impair wing disc regeneration, and that local knockdown of the Ec-degradation enzyme *cyp18a1* reciprocally enhances regeneration. In parallel, we find that injury induces a transient ring of Ec receptor (EcR) activity encircling the regenerating blastema which can be blocked by *phm* knockdown. In the absence of this EcR-activity pulse, we find a failure of intra-organ growth inhibition as cells within the uninjured compartments of the wing disc fail to slow their proliferation. Further, injured animals with *phm* knockdown lack a robust injury-induced developmental delay, while *cyp18a1* knockdown has the opposite effect of extending injury-induced developmental delay. We will discuss mechanistic links between local Ec/EcR and signals produced by injured tissues that act systemically to control tissue homeostasis.

98 Blocking the native differentiation program recapitulated in *yki*$^{3S/A}$-induced midgut tumor alters the tumor cells' capacity to disseminate and induce cachexia-like wasting *Inez Pranoto*, Young Kwon University of Washington, Seattle WA

The developmental program generates heterogeneous cell types that form and maintain tissue structure and physiology. Many tumors have been shown to recapitulate the developmental program of the tissue of origin, thus generating heterogenous cells within the tumors. Although tumor cells with stem cell-like properties have been extensively studied, how developmental programs recapitulated in tumors or tumor cells undergoing differentiation contribute to the adverse phenotypes associated with malignant tumors, such as metastasis and cachexia, are only poorly understood. We employed *Drosophila* midgut tumors driven by expression of the active form of *yorkie* (*yki*$^{3S/A}$) and revealed the striking heterogeneity of *yki*$^{3S/A}$ midgut tumor cells, which aberrantly reconstituted the differentiation program to generate enterocytes (ECs)– polyploid absorptive intestinal epithelial cells. Blocking the EC differentiation program by expressing dominant negative Notch (N^{DN}) significantly reduced the heterogeneity of *yki*$^{3S/A}$ tumor cells by eliminating the differentiating *yki*$^{3S/A}$ tumor cells, leading to formation of fully grown tumors comprising only intestinal stem cell-like *yki*$^{3S/A}$ tumor cells. Of note, blocking the EC differentiation program was sufficient to eliminate a population of *yki*$^{3S/A}$ tumor cells that can invade and migrate to disseminate from the midguts. Interestingly, eliminating *yki*$^{3S/A}$ tumor cells undergoing EC differentiation significantly altered the expression of multiple tumor-driven secreted factors and the tumor's propensity for inducing the tumor non-autonomous and systemic phenotypes. In particular, blocking the EC differentiation program significantly decreased the expression of wasting factors in tumors and suppressed multiple wasting phenotypes, including ovary atrophy, fat body degeneration, and 'bloating syndrome'. Our study demonstrates that the EC differentiation program recapitulated in *yki*$^{3S/A}$ midgut tumors is crucial for the tumor cells'

capacity to disseminate from the midguts and induce certain wasting phenotypes. This work provides insights into how the contextual information in the tissue of origin can give rise to tumor's ability to induce the phenotypes associated with malignant tumors. Thus, we propose that manipulating the developmental/differentiation programs recapitulated in tumors is a way to modulate tumor behavior for the treatment of the complications associated with the advanced cancers.

99 Enterocyte dynamics in the *Drosophila* adult midgut epithelium upon infection *Shyama Nandakumar*[1,2], NIcolas Buchon[1,2] 1) Cornell Institute of Host-Microbe Interactions and Disease, Cornell University; 2) Department of Entomology, Cornell University

Enterocytes are a well conserved cell type and make up the majority of the digestive system across metazoans. In *Drosophila*, enterocytes comprise nearly 90% of the adult gut epithelium, and play important roles in digestion such as secretion of digestive enzymes and absorption of nutrients, maintaining gut epithelial barriers and regulating tissue turnover. Enterocytes are also the first line of defense against oral pathogens. However, despite their critical functions, enterocytes are not as well studied as the other cells in the midgut epithelium. Enterocytes are also subject to frequent cell turnover in the midgut epithelium, a process that is acutely accelerated upon infection. What determines the decision of an enterocyte to remain in the epithelium or to delaminate? To understand this, we have developed a number of genetic tools as well as microscopy and flow cytometry based assays to study epithelial dynamics and cell turnover in the midgut over a comprehensive time course following oral infection with multiple pathogens such as *Erwinia carotovora 15 (Ecc15)* and *Pseudomonas entomophilia (Pe*). We have identified key differences between the anterior and posterior midgut in the kinetics and amplitude of cell loss and enterocyte turnover upon infection in both male and female adult flies. We observe differences in enterocyte loss in response to infection with *Ecc15* and *Pe*. At the tissue level, we have identified mechanical changes that the midgut epithelium undergoes in response to oral pathogens such as muscle constrictions, gut contraction and region-specific changes in length. Additionally, at the cellular level, we have identified acute and dynamic changes in enterocyte cell shape, size and the nature of cell-cell junctions. We are currently investigating the genetic factors responsible for these changes, and how they impact gut physiology and infection response in the adult fly. We are also employing live imaging strategies to monitor enterocyte dynamics *in vivo* under various conditions. Our work will provide several key insights into the biology of enterocytes under physiological as well as pathological conditions.

100 PAAC to the new normal: Intravital imaging of dynamic brush border repair in the adult *Drosophila* intestine *Anthony Galenza*[1], Yu-Han Su[1], Paola Moreno-Roman[1], Irina Kolotuev[2], Lucy O'Brien[1] 1) Stanford University, Stanford, CA; 2) Universite de Lausanne, Lausanne, Switzerland

Frequent exposure of the intestine to hostile pathogens and harsh toxins demands rapid and seamless repair. Because the intestine is located inside the body cavity, examination of the intestine's physiological repair processes has traditionally been limited to fixed tissues. Here we use an intravital imaging methodology, Bellymount, to monitor real-time intestinal injury and subsequent repair in individual animals over multiple days. Within 24 hours of toxin feeding, components of the intestinal brush border, such as Moesin::GFP and Meduse::GFP, relocalize from lumenal cell surfaces to lateral cell surfaces; after 48 hours, they disappear entirely. Concomitant with loss of the intestinal brush border, Moesin::GFP and Meduse::GFP appear in nascent brush border membranes associated with new stem cell progeny. Unlike new brush border formation during normal intestinal renewal, which involves a strikingly large, Pre-Assembled Apical Compartment (PAAC) at tri-cellular junctions (Moreno-Roman *et al.*, *bioRxiv* 457819), new brush border formation during repair involves linear clusters of 4-8 miniature PAACs at bi-cellular junctions. We call these clusters multi-PAACs. Either genetic ablation of the brush border in mature cells or activation of the injury response pathway JAK-STAT in stem cell progeny is sufficient to induce multi-PAACs in otherwise healthy guts. These findings delineate how damage signals trigger an injury-specific morphogenetic process. Together, these results reveal spatiotemporal dynamics of brush border damage and regeneration and provide insights into fundamental mechanisms of epithelial barrier repair.

101 Asymmetric nucleosome density and differential condensation of sister chromatids coordinates with Cdc6 to ensure distinct cell fates *Rajesh Ranjan*[1], Xin Chen[1, 2] 1) Johns Hopkins University,; 2) Howard Hughes Medical Institute (HHMI)

Stem cells undergo asymmetric division to produce both a self-renewing stem cell and a differentiating daughter cell. During *Drosophila* male germline stem cell (GSC) asymmetric division, preexisting old histones H3 and H4 are enriched in the self-renewed stem daughter cell, whereas the newly synthesized H3 and H4 are enriched in the differentiating daughter cell. However, the biological consequences in the two daughter cells resulting from asymmetric histone inheritance remained to be elucidated. In this work, we track both old and new histones throughout GSC cell cycle using high spatial and temporal resolution microscopy. We find several unique features differentiating old versus new histone-enriched sister chromatids, including nucleosome density, chromosomal condensation, and H3 Ser10 phosphorylation. These distinct chromosomal features lead to their differential association with Cdc6, an essential component of the pre-replication complex, which subsequently contributes to asynchronous initiation of DNA replication in the two

resulting daughter cells. Disruption of asymmetric histone inheritance abolishes both differential Cdc6 association and asynchronous S-phase entry, demonstrating that asymmetric histone acts upstream of these critical events during cell cycle progression. Furthermore, GSC defects are detected under these conditions, indicating a connection between histone inheritance, cell cycle progression and cell fate decision. Together, these studies reveal that cell cycle remodeling as a crucial biological 'readout' of asymmetric histone inheritance, which precedes and could lead to other well-known readouts such as differential gene expression. This work also enhances our understanding of asymmetric histone inheritance and epigenetic regulation in other stem cells or asymmetrically dividing cells in multicellular organisms.

102 Rab35 mediates two distinct pathways that regulate actin modification through Mical/SelR and actin remodeling through Septins during cell wound repair *Mitsutoshi Nakamura*, Justin Hui, Tessa Allen, Susan M. Parkhurst Basic Sciences Division, Fred Hutchinson Cancer Research Center, Seattle, WA

Cell wound repair is essential to cope with daily wear and tear, especially when cells are fragile as a result of trauma or from disease conditions. The three main steps of wound repair are highly conserved among different organisms: rapid membrane resealing; dynamic cytoskeletal reorganization for wound closure; and cell cortex (membrane and underlying cytoskeleton) remodeling to restore the cell to its unwounded state. We recently found that Rab35 is required for the cell cortex remodeling phase following wound closure in the *Drosophila* cell wound repair model. Rab35, a member of Rab family GTPases, regulates membrane trafficking and cytoskeleton dynamics in many cellular processes such as cytokinesis. We find that Rab35 is recruited to wounds and its RNAi knockdown exhibits premature actomyosin ring disassembly. Interestingly, we find that Rab35 functions in this context through two different downstream pathways. One pathway involves Mical, an oxidation-reduction enzyme that binds and disassembles F-actin, and SelR, a methionine sulfoxide reduction enzyme that works as a counterpart to Mical. Both Mical and SelR are recruited to wounds and their RNAi knockdowns exhibit opposite phenotypes in actomyosin ring remodeling. The other pathway involves Septins, a family of GTP-binding proteins that form hetero-oligomeric structures at the cell cortex to regulate cytoskeleton. We find that all five *Drosophila* Septins are recruited to wounds with similar, but not identical, spatial and temporal patterns. Consistent with this, they exhibit different mutant phenotypes with respect to actin dynamics, suggesting that Septins have non-redundant and complex-independent functions during cell wound repair. We are currently investigating the coordination between these pathways needed for proper remodeling of the cell cortex upon wound closure.

103 Re-entry into mitosis and regeneration of intestinal stem cells through enteroblast dedifferentiation in *Drosophila* midguts *Aiguo Tian*[1], Virginia Morejon[1], Sarah Kohoutek[1], Yi-Chun Huang[1], Wu-Min Deng[1], Jin Jiang[2] 1) Tulane University; 2) UT Southwestern Medical Center

Colorectal cancer (CRC) is the 3rd most common cancer diagnosed in the United States. Intestinal dysplasia is considered as a precursor lesion of CRC in mammals and can be mimicked in *Drosophila*. Dysplasia is characterized by hyperproliferation and accumulation of intestinal stem cells (ISCs) and enteroblasts (EBs). As a result of dysplasia, the lifespan is shortened. Current studies in *Drosophila* indicate that only resident ISCs can divide and their over-proliferation results in intestinal dysplasia. In addition, enteroblasts (EBs) as one type of ISC progenies are accumulated under intestinal dysplasia. Thus, our studies use the *Drosophila* intestine as a model to understand how EBs contribute to dysplasia in response to infection of pathogenic bacteria. Here we find that infection of pathogenic bacteria induces enteroblasts (EBs) as one type of ISC progenies to re-enter the mitotic cycle in the *Drosophila* intestine. The re-entry into mitosis is dependent on epithermal growth factor receptor (EGFR)-Ras signaling and ectopic activation of EGFR-Ras signaling in EBs is sufficient to drive EBs cell-autonomously to re-enter into mitosis. In addition, we examined whether EBs gain ISC identity as a prerequisite to divide, but the immunostaining with stem cell marker Delta shows that these dividing EBs do not gain ISC identity. After employing lineage tracing experiments, we further demonstrate that EBs dedifferentiate to generate functional ISCs after symmetric divisions of EBs. Together, our study in *Drosophila* intestines uncovers a new role of EGFR-Ras signaling in regulating re-entry into mitosis and dedifferentiation during regeneration and reveals a novel mechanism by which ISC progenies undergo dedifferentiation through a mitotic division, which has important implication to increase of ISC pool and intestinal dysplasia.

104 Cellular and molecular basis of detection of acidic pH in fly gustatory system *Anindya Ganguly*[1], Avinash Chandel[1], Shan Wang[1], Heather Turner[2], Emily Liman[2], Craig Montell[1] 1) University of California, Santa Barbara; 2) University of Southern California

Drosophila melanogaster use their sense of taste to forage for food, avoid toxic or hazardous substances, to select partners for courtship and mating and to choose suitable oviposition sites. Although the cellular and molecular basis of sweet, bitter and salt tastes have been well characterized in flies a detailed understanding of how they sense acid is lacking. Based on past studies, members of Gustatory Receptor (GR) or Ionotropic Receptor (IR) families are unlikely to function broadly as acid receptors. Hence, we elected to test whether fly genes that are distantly related to mammalian Otop1, a proton channel required for sour taste in mammals, are required for acid taste in flies. RNAi-mediated silencing as well as CRISPR/Cas9-mediated mutagenesis of *OtopLA*, but not of *OtopLB* or of *OtopLC* caused a severe reduction in behavioral aversion to sweet solutions in the presence of low pH or high concentrations of carboxylic acids as well as

action potentials induced by acids on neurons in labellar taste bristles. We identified a novel *OtopLA* isoform from the proboscis (*OtopLAp*)that rescued the behavioral deficit as well as the reduced neuronal sensitivity to acids exhibited by the *OtopLA* mutant. Although high concentrations of HCl caused behavioral aversion, we observed mild attraction to low concentrations of HCl. This attraction was also impaired in *OtopLA* mutant. By conducting cell-type specific genetic rescue experiments in various subsets of taste neurons, we demonstrated that *OtopLA* functions in different neuronal subsets to cause different behavioral outcomes. This study demonstrates functional conservation of a taste receptor between flies and mammals. It also elucidates that the same taste receptor is required both for appetitive and repulsive gustatory behaviors.

105 Developmental mechanisms regulating the formation and function of drosophila sleep-wake circuit Adil R Wani[1], Aisha Hamid[1], Hannah Deutsch [2], Gonzalo M Chaya[1], Matthew S Kayser[2], *Mubarak H Syed*[1] 1) University of New Mexico, Albuquerque, NM; 2) University of Pennsylvania, PA

Molecular mechanisms regulating the formation and function of neural cell types are not fully understood. Studying the programs by which neuronal types are specified, connected to form unique functional circuits regulating discrete behaviors is an important area of neuroscience. We are investigating this long-standing question using the recently identified and conserved Drosophila sleep-wake circuit. We utilize novel lineage filtering tools to show that sleep-promoting dorsal fan-shaped body (dFB) neurons are born from late-type II neural stem cells (NSCs) that express ecdysone induced protein (E93). Using mosaic analysis, we show that majority of the dFB neurons are generated from the dorsolateral 1 (DL1) type II NSCs, and E93 is essential for the specification of these neurons. Interestingly, the knockdown of E93 in type II NSCs affects adult sleep behavior. Our data show that we have identified steroid hormone-mediated NSC-specific developmental programs that regulate the formation and function of the sleep-wake circuit.

106 Associative learning drives longitudinally-graded presynaptic plasticity of neurotransmitter release along axonal compartments *Aaron Stahl*[1], Nathaniel Noyes[1], Tamara Boto[2], Miao Jing[3], Jianzhi Zeng[4,5,6], Lanikea King[1], Yulong Li[3,4,5,6], Ronald Davis[1], Seth Tomchik[1] 1) The Scripps Research Institute, Jupiter, FL; 2) Trinity College, Dublin, Ireland ; 3) Chinese Institute for Brain Research, Beijing, China; 4) State Key Laboratory of Membrane Biology, Peking University School of Life Sciences, Beijing, China; 5) Peking-Tsinghua Center for Life Sciences, Beijing, China; 6) PKU- IDG/McGovern Institute for Brain Research, Beijing, China

Anatomical and physiological compartmentalization of neurons is a mechanism to increase the computational capacity of a circuit, and a major question is what role axonal compartmentalization plays. Axonal compartmentalization may enable localized, presynaptic plasticity to alter neuronal output in a flexible, experience-dependent manner. Here we show that olfactory learning generates compartmentalized, bidirectional plasticity of acetylcholine release that varies across the longitudinal compartments of *Drosophila* mushroom body (MB) axons. The directionality of the learning-induced plasticity depends on the valence of the learning event (aversive vs. appetitive), varies linearly across proximal to distal compartments following appetitive conditioning, and correlates with learning-induced changes in downstream mushroom body output neurons (MBONs) that modulate behavioral action selection. Potentiation of acetylcholine release was dependent on the $Ca_V2.1$ calcium channel subunit *cacophony*. In addition, contrast between the positive conditioned stimulus and other odors required the inositol triphosphate receptor (IP_3R), which was required to maintain responsivity to odors in untrained conditions. These data demonstrate that learning drives valence-correlated, compartmentalized, bidirectional potentiation and depression of synaptic neurotransmitter release, which rely on distinct mechanisms and are distributed across axonal compartments in a learning circuit.

107 A conserved RNA binding protein regulates RNAs critical for neurodevelopment *Carly Lancaster*, Ken Moberg, Anita Corbett 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 trafficking and translation. Our labs co-discovered a monogenic form of ID caused by loss-of-function mutations in the ubiquitously expressed RBP ZC3H14. Studies exploiting the conserved ZC3H14 ortholog in *Drosophila*, Nab2, reveal 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 m6A and Nab2 in

controlling Trio splicing and expression, along with Nab2-Trio coregulation of axonal development in the CNS. 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.

108 Recovery from cold-induced reproductive dormancy is regulated by temperature-dependent AstC signaling *Matthew R. Meiselman*[1], Michael H. Alpert[2], Xinyue Cui[1], Jamien Shea[1], Ian Gregg[1], Marco Gallio[2], Nilay Yapici[1] 1) Department of Neurobiology and Behavior, Cornell University, Ithaca, NY, 14853, USA; 2) Department of Neurobiology, Northwestern University, Evanston, IL, 60208, USA

Many species have evolved a variety of behaviors to cope with adverse environmental conditions. Similar to many insects, *Drosophila melanogaster* responds to sustained cold temperatures by reducing locomotion, metabolic rate, and arresting reproduction. Here, we show that a subset of dorsal neurons (DN3s) that express the neuropeptide Allatostatin-C (AstC) in the fly brain facilitates recovery from cold-induced reproductive dormancy. Activity of AstC-expressing DN3s is modulated by temperature and AstC peptide levels are suppressed at cold temperatures in *Drosophila* and in vector mosquitoes. The stimulatory effect of AstC on egg production during and after cold-induced dormancy is meditated by AstC-R2-expressing CCHA2 neurons, whose activation is sufficient to induce reproductive dormancy in warm temperatures. Our results demonstrate that DN3s coordinate female reproductive capacity with environmental temperature via AstC signaling. AstC/AstC-R2 is conserved across many insect species and their role in regulating female reproductive capacity makes them an ideal target for controlling the population of agricultural pests and human disease vectors.

109 Orion bridges phosphatidylserine and Draper in the phagocytosis of somatosensory neurons in *Drosophila* *Hui Ji*[1], Bei Wang[1], David Labib[1,3], Joyce Lei[1,4], Xinchen Chen[1], Maria Sapar[1,3], Ana Boulanger[2], Jean-Maurice Dura[2], Chun Han[1] 1) Weill Institute for Cell and Molecular Biology and Department of Molecular Biology and Genetics, Cornell University, Ithaca, NY ; 2) IGH, Centre National de la Recherche Scientifique, Université de Montpellier, Montpellier, France; 3) New York Stem Cell Foundation, New York, NY ; 4) Tisch MS Research Center of New York, New York, NY

Phagocytic clearance of degenerating neurites is critical for the prevention of neuroinflammation and neuronal regeneration. Phosphatidylserine (PS), an 'eat-me' signal exposed on degenerating neurites, and Draper (Drpr), an engulfment receptor expressed by phagocytes, are known to mediate the phagocytosis of degenerating neurites in *Drosophila*. However, how PS is sensed by Drpr-expressing phagocytes remains poorly understood. A novel secreted protein, Orion, was recently found to be required for axon pruning of mushroom body neurons during metamorphosis. In this study, we investigated whether Orion functions as a bridging molecule between PS and Drpr using larval dendritic arborization (da) neurons and phagocytic epidermal cells as a model of neuronal phagocytosis. First, using multiple models of dendrite degeneration, we demonstrate that Orion is required for Drpr-mediated phagocytosis of dendrite debris. *orion* loss-of-function (LOF) did not interfere with PS exposure on dendrites but abolished recruitment of epidermal Drpr to degenerating dendrites, suggesting that Orion acts downstream of neuronal PS exposure and upstream of Drpr. Second, we found that Orion is secreted by many non-neural tissues and shows PS-dependent binding to degenerating dendrites. Orion outcompetes Annexin V in binding to injured dendrites, suggesting a direct interaction with PS. Third, we found that Orion functionally interacts with Drpr: Orion accumulates on epidermal cells that overexpressed Drpr. Importantly, a membrane-tethered Orion induced PS-independent and Drpr-dependent dendrite degeneration when expressed in neurons but blocked engulfment of dendrites when expressed in epidermal cells. Lastly, we found that the Orion dosage modulates the sensitivity of the epidermal cell to PS: While *orion* heterozygosity reduced epidermal engulfment of PS-exposing dendrites, Orion overexpression caused engulfment-dependent, ectopic degeneration of wildtype dendrites.
Together, these findings argue that Orion functions as a bridging molecule between PS and Drpr and fine-tunes the competence of phagocytes in neuronal engulfment. The shared features between Orion and human chemokines that were recently demonstrated to function as "find-me" signals by binding to PS-exposing vesicles suggest conserved mechanisms between insects and humans in phagocytosis and homeostatic regulations of diverse body systems.

110 The Circular RNA *Edis*-Relish-Castor Axis Regulates Neurodevelopment *Wei Liu*[1], Weihong Liang[1], Xiao-Peng Xiong[2], Jian-Liang Li[3], Rui Zhou[1] 1) Depts. of Medicine, Biological Chemistry & Oncology, Johns Hopkins University School of Medicine, Baltimore, MD ; Johns Hopkins All Children's Hospital, St. Petersburg, FL ; 2) Sanford Burnham Prebys Medical Discovery Institute, La Jolla, CA ; 3) NIEHS, Durham, NC

We have identified and validated a collection of circular RNAs in *Drosophila melanogaster* in response to immune challenge. We show that depletion of the brain-enriched circular RNA *Edis* (*Ect4-d*erived *i*mmune *s*uppressor), but not its linear sibling *Ect4*, causes hyperactivation of antibacterial innate immunity signaling both in cultured cells and *in vivo*. Conversely, ectopic expression of *Edis* blocks innate immunity signaling. Our analyses reveal that *Edis* is enriched in neurons, and that neuron-specific depletion of *Edis* leads to defective axon guidance, impaired locomotive activity and shortened lifespan. Importantly, restoration of *Edis* expression suppresses the innate immunity hyperactivation

and neurodevelopment phenotypes elicited by *Edis* depletion. Mechanistically, *Edis* encodes a functional protein that associates with and blocks the processing/activation of the innate immunity transcription factor Relish. We further show that the *Castor* gene, which encodes a transcription factor involved in neurodevelopment, is upregulated in *Edis* knockdown neurons. Notably, *Castor* overexpression phenocopies *Edis* overexpression in neurons, and reducing *Caster* levels suppresses the neurodevelopmental phenotypes in *Edis* knockdown neurons. Chromatin immunoprecipitation assay reveals that Relish associates with the promoter of *Castor*. In addition, we show that Relish-binding sites are required for optimal *Castor* promoter activity induced by either peptidoglycan treatment or *Edis* depletion. Lastly, *Relish* mutation rescues both *Castor* hyperactivation and neurodevelopmental phenotypes in *Edis* knockdown neurons. We conclude that the circular RNA *Edis* serves as a key modulator of innate immunity, and that the *Edis*-Relish-Castor axis regulates neurodevelopment.

111 Netrins and receptors control *Drosophila* optic lobe organization and transmedullary neuron axon targeting *Yu Zhang*, Scott Lowe, Xin Li The School of Molecular and Cellular Biology

During development, integration of temporal patterning and spatial patterning of neural progenitors as well as Notch-dependent binary fate choice between sister neurons contribute to generation of neural diversity. How these upstream neural fate specification programs regulate downstream effector genes to control axon targeting and neuropil assembly remains less well-understood. Here we show that Notch-dependent binary fate choice in *Drosophila* medulla neurons regulates the expression of Netrin, and that Netrin pathway controls axon guidance of transmedullary (Tm) neurons and contributes to the organization of optic lobe neuropils. Netrins are enriched in the lobula where Tm axons target, and the attractive receptor Frazzled is expressed broadly in medulla neurons, while the repulsive receptor Unc-5 is excluded from Tm neurons and this is necessary for their correct targeting to the lobula. Frazzled is required collectively in a group of early-born Tm neurons for their axons to establish the inner optic chiasm (IOC). In addition, Frazzled acts in the layer-specific targeting step of Tm3 and Tm4 cell-autonomously, and is also required for the formation of the lobula branch of TmY3. Moreover, we show that the diffusibility of Netrins is necessary for Netrin enrichment in the lobula, the IOC formation and layer-specific targeting of Tm3 and Tm4. Netrin enrichment in the lobula is promoted by Frazzled expressed in Tm neurons, while Unc-5 appears to have an opposite role in Netrin distribution. Furthermore, we show that Netrin B is expressed in the Notch-on hemilineage of medulla neurons including most Tm and TmY neurons that target lobula, and loss of Su(H) abolished NetB expression in the medulla. Without medulla-originated NetB, Tm axons from late-born medulla columns cannot join the IOC. Therefore, the Notch-dependent binary fate choice regulates the assembly of the optic lobe neuropils by controlling the expression of Netrin.

112 Functional divergence of the *bag of marbles* gene in the *Drosophila melanogaster* species group *Jaclyn Bubnell*, Cynthia Ulbing, Paula Fernandez Begne, Charles Aquadro Cornell University

In *Drosophila melanogaster,* a key germline stem cell (GSC) differentiation factor, *bag of marbles* (*bam*) shows rapid bursts of amino acid fixations between sibling species *D. melanogaster* and *D. simulans*, but not in the outgroup species *D. ananassae*. We previously hypothesized that a change in function and/or a genetic conflict with the intracellular bacteria *W. pipientis* could be driving the adaptive evolution of *bam*, however *bam* function has only been defined in *D. melanogaster*. Here, we test the null hypothesis that *bam'*s differentiation function is conserved in four additional *Drosophila* species in the *melanogaster* species group spanning approximately 15 million years of divergence. Surprisingly, we demonstrate that *bam* is not necessary for oogenesis or spermatogenesis in *D. teissieri* nor is *bam* necessary for spermatogenesis in *D. ananassae*. Remarkably *bam* function may change on a relatively short time scale. We further report tests of neutral sequence evolution at *bam* in additional species of *Drosophila* and find a positive correlation between evidence for positive selection at *bam* and its essential role in GSC regulation and fertility for both males and females. Further characterization of *bam* function in more divergent lineages will be necessary to distinguish between *bam's* critical gametogenesis role being newly derived in *D. melanogaster*, *D. simulans* and *D. yakuba* or it being basal to the genus and subsequently lost in numerous lineages.

113 Cross-species incompatibility between a DNA satellite and the Drosophila homolog of Spartan poisons germline genome integrity *Cara Brand*, Mia Levine University of Pennsylvania

Satellite DNA spans megabases of eukaryotic genome sequence. These vast stretches of tandem DNA repeats undergo high rates of sequence turnover, resulting in radically different satellite DNA landscapes between closely related species. Such extreme evolutionary plasticity suggests that satellite DNA accumulates mutations with no functional consequence. Paradoxically, satellite-rich genomic regions support essential, conserved nuclear processes, including chromosome segregation, dosage compensation, and nuclear structure. A leading resolution to this paradox is that deleterious proliferation of satellite DNA variants trigger adaptive evolution of satellite-associated chromosomal proteins to preserve these essential functions. Here we experimentally test this model of intra-genomic coevolution between chromosomal proteins and DNA satellites by conducting an evolution-guided manipulation of both. The 359bp satellite spans an 11Mb array in *D. melanogaster* that is absent from its sister species, *D. simulans*. This species-specific satellite array colocalizes with the Drosophila protein, Maternal Haploid (MH), an essential, adaptively evolving member of the

Spartan family of proteases that cleave DNA-protein crosslinks. To determine if MH and 359 coevolve, we swapped the *D. simulans* version of MH ("MH[sim]") into *D. melanogaster*. We discovered that MH[sim] triggers elevated ovarian cell death, reduced ovary size, and loss of mature eggs. In contrast, *mh* null females have no such ovary phenotypes, suggesting that MH[sim] is toxic. Using both cell biological and genetic approaches, we demonstrate that MH[sim] poisons oogenesis through a DNA damage pathway. Remarkably, deleting the *D. melanogaster*-specific 359 satellite array from *mh[sim]* females completely restores female germline genome integrity and fertility, consistent with a history of coevolution between these two fast-evolving components of the Drosophila genome. Genome integrity and female fertility are also restored by overexpressing Topoisomerase II (Top2). Top2 resolves DNA entanglements by generating transient double stranded breaks while crosslinked to DNA. Intriguingly, these DNA-Top2 crosslinks are resolved by the MH homolog in worms and humans. We propose that MH[sim] prematurely cleaves DNA-Top2 crosslinks that form during Top2-mediated processing of 359 entanglements. Under this model, MH evolved adaptively along the *D. melanogaster* lineage to avoid 359 in the ovary, leading to the observed cross-species incompatibility between the 359 satellite and MH. Our study offers rare experimental evidence that intra-genomic coevolution between ostensibly inert repetitive DNA and essential chromatin proteins preserves germline genome integrity.

114 A putative *de novo* evolved gene is essential for male fertility via a paternal effect *Sara Guay*, Jill O'Toole, Prajal Patel, Geoffrey Findlay College of the Holy Cross, Worcester, MA, USA

De novo evolved genes arise from previously non-coding DNA regions and are found in only one or a few species. In *D. melanogaster*, many *de novo* genes are expressed in the testis, suggesting they may affect male fertility and be subjected to sexual selection. An RNAi and CRISPR knockout screen identified *katherine johnson* (*kj*) as one of several putative *de novo* genes essential for male fertility. Males knocked down or knocked out for *kj* show a 90% reduction in fertility. Curiously, dissection of mutant testes revealed high numbers of mature sperm in the seminal vesicle. Furthermore, these sperm are transferred to females and enter the seminal receptacle at rates equivalent to sperm of controls. Instead, measurements of egg-to-adult viability showed that the loss of *kj* causes a fertility defect because of a failure of embryos fathered by *kj* null males to develop into larvae. Preliminary evidence indicates that embryos fathered by *kj* nulls initiate mitotic divisions, confirming that *kj* null male sperm are fertilization competent. However, by 6 hours after fertilization, embryos of *kj* null males show a significantly reduced rate of developmental progression. To understand the normal function of the KJ protein, and how its loss causes this developmental defect, we constructed a transgenic fly line expressing a 6xHis-tagged *kj* transgene under the control of its own regulatory sequences. We are currently examining KJ-HA localization and further evaluating the exact stage of embryonic development at which *kj* null-fathered embryos begin to show defects. While several other putative *de novo* genes are required for successful sperm production, *kj* represents the first such gene to our knowledge to be required for the proper use of the paternal genome after fertilization. These findings further establish the ability of newly evolved genes to evolve essential roles in reproduction.

115 The Y-linked gene, *WDY*, is necessary for sperm storage in *Drosophila melanogaster*. *Yassi Hafezi*, Arsen Omurzakov, Mariana Wolfner, Andrew Clark Cornell University

Only 13 single-copy protein-coding genes are known on the *Drosophila melanogaster* Y-chromosome -- a chromosome that is 40 Mb, haploid, paternally inherited, entirely heterochromatic, repeat-dense, and lacks recombination. These genes display remarkable "functional coherence," with five Y-linked genes (three of which encode dynein motor components) being necessary to produce mature sperm past the early individualization stage of spermatogenesis. The significance of placing these fertility-essential genes in a heterochromatic and degenerating region of the genome is not understood. We are now discovering additional, more subtle functions associated with the Y chromosome. We and others recently demonstrated by knockdown that the gene, *WDY*, is responsible for the sterility of the *kl-1* region of the Y chromosome. Here we further investigate the phenotype of *WDY* using CRISPR mutants we generated. Using compound sex chromosomes, we established several stable mutant lines for *WDY*. Mutant males were sterile, as expected, but surprisingly produced mature, motile sperm. *WDY* mutant sperm were transferred to females, albeit at lower rates. Consistent with prior reports associated with the *kl-1* region, we found that *WDY* mutant sperm are unable to enter storage in the female. This failure may be caused by a motility defect of the sperm or an inability to interact with female molecules that enable storage. Further analysis of *WDY* may reveal mechanisms by which sperm are stored, as well as clues to the underlying evolutionary logic of locating a gene on the Y chromosome.

116 Investigating the evolution of new body parts in the rapidly evolution genitalia of Drosophila *Gavin Rice*[1], Kenechukwu Charles-Obi[1], Jean David[2], Nicolas Gompel[3], Amir Yassin[2,4], Julia Zeitlinger[5,6], Mark Rebeiz[1] 1) Department of Biology, University of Pittsburgh, Pittsburgh, PA; 2) Laboratoire Evolution, Génomes, Comportement, Ecologie (EGCE), UMR 9191, CNRS,IRD, Univ.Paris-Sud, Université Paris-Saclay, cedex, France; 3) Ludwig-Maximilians Universität München, Fakultät für Biologie, Biozentrum, Grosshaderner Strasse 2, 82152 Planegg-Martinsried, Germany; 4) Institut de Systématique, Evolution et Biodiversité, UMR7205, Centre National de la Recherche Scientifique, MNHN, Sorbonne Université, EPHE, Université des Antilles, 57 rue Cuvier, 75005 Paris, France ; 5) Stowers Institute for Medical Research, Kansas City, MO; 6) Department of Pathology and Laboratory Medicine, Kansas University Medical Center, Kansas City,

MO

Recently evolved traits i.e., novelties, often represent key features that allow animals to exploit new ecological niches (e.g. feathers in birds) and can even help them find a mate (e.g. bioluminescence in fireflies). The rapidly evolving genitalia of Drosophila provides a powerful system to study the developmental basis of qualitative changes in morphology. However, this high morphological diversity also poses a distinct challenge, since it can be difficult to disentangle which structures are homologous and which represent novelties.

To determine whether genital structures of different species were homologous or novel, we compared the development of phallic outgrowths in the Oriental lineage, which contains *Drosophila melanogaster*. We found that most phallic outgrowths are formed by **multicellular** projections. However, several multicellular outgrowths that were thought to be homologous, are in fact formed by different tissues of the phallus in different species, indicating that they are heterologous structures. Furthermore, we uncovered evidence that phallic outgrowths found in the *Drosophila eugracilis* phallus are formed by large **unicellular** projections, suggesting that they have evolved convergently to the previously described multicellular outgrowths. These unicellular outgrowths are likely novel as they are absent the homologous tissue of five other species of the Oriental lineage. We found that the trichome genetic network is expressed in these unicellular phallic outgrowths, suggesting that the co-option of this network underlies this dramatic phenotype. In fact, activation of the trichome genetic network in the phallus of *Drosophila melanogaster* induces a phenocopy of the unicellular outgrowths found in *Drosophila eugracilis*.

Our work highlights that understanding development can be key to discern between homology vs heterology, allowing us to formulate testable evolutionary models (i.e. co-option vs convergence).

117 Do supergenes mediate seasonal adaptation in overwintering *Drosophila*? *Joaquin Nunez*[1], Alyssa Bangerter[1], Connor Murray[1], Yang Yu[1], Benedict Lenhart[1], Priscilla Erickson[1,2], Alan Bergland[1] 1) University of Virginia, Charlottesville, VA; 2) University of Richmond, Richmond, VA

Vinegar flies (*Drosophila melanogaster*) living in temperate regions experience strong fluctuations in the strength and direction of selection due to seasonality and evolve rapidly to track the changing fitness landscape. Recent work has shown the existence of genomic loci whose seasonal signal is replicated across continents. Despite these advances in identifying the presence of fluctuating selection and adaptation between seasons, we still lack a detailed understanding of the functional genetic architecture of seasonal adaptation. This gap in knowledge is further compounded by the fact that wild *Drosophila* populations experience demographic “boom-and-bust” cycles concomitant with seasonality, a process which may alter levels of standing genetic variation. We characterized both the genomic consequences of boom-and-bust cycles as well as the targets of seasonally varying selection. We accomplished this by leveraging temperature data combined with genomic sequences from densely sampled orchard populations collected within (June-November) and across growing seasons over multiple populations on two continents. Principal component analysis reveals temporal structure, and using simulation, we show that this temporal structure is consistent with cyclical winter population collapse. This temporal signal is observed genome-wide except for chromosome 2L, where allele frequencies of the cosmopolitan inversion *Inv(2L)t* drive genetic structure. We further investigated these patterns at *Inv(2L)t* by regressing the mean temperature, 30 days prior to sample collection, onto allele frequencies of each SNP using a generalized linear model. This analysis reveals an enrichment of loci responding to temperature clustered within *Inv(2L)t.* Linkage analysis suggest that the loci associated with temperature changes lie within the inversion but is not the inversion *per se*. Additional analyses show multiple mutations in high linkage at distances ~0.1 – 0.5 Mb, suggesting long range associations among seasonal loci inside the inversion. Enrichment analysis show an excess of non-synonymous mutations among the *Inv(2L)t* seasonal outliers. Overall, our data provides two insights about *Drosophila* seasonality. First, we show that severe demographic contractions and expansions are an important, and measurable, component of the seasonal dynamics of temperate *Drosophilids*. Second, our data supports the general hypothesis that supergenes are important drivers of adaptation in highly fluctuating environments.

118 The evolution and genetic mechanism of sex-ratio meiotic drive in *Drosophila affinis* *Wen-Juan Ma*, Robert L. Unckless University of Kansas

Meiotic drivers are selfish genetic elements that promote their own transmission to the gametes. Sex-ratio (SR) meiotic drive occurs when a selfish genetic element on the X chromosome manipulates gametogenesis to prevent the maturation of Y-bearing sperm in males, resulting in the production of predominant female progeny. The spread of SR meiotic drive can affect host genetic diversity, sex chromosome evolution, and even cause host extinction if it reaches high enough frequency. SR meiotic drivers have evolved independently several times, however, the underlying genetic mechanism is only known in few cases. In this study we use a combination of genomics, transcriptomics, single nuclei RNA-seq (snRNA-seq), and CRISPR-Cas9 to identify the genetic mechanism responsible for the SR meiotic drive in *Drosophila affinis*. We found X chromosome is enriched for differentially expressed (DE) transcripts and that many DE genes had elevated Ka/Ks values between SR and ST. We identified top DE candidate genes, including two X-linked

duplicate and testis-specific: encoding a chromosomal protein involved in spermatogenesis (*tHMG2*), and encoding the regulator of chromatin condensation (*RCC1*). We used CRISPR-Cas9 knockout experiments to test whether these genes were involved in the sex ratio phenotype. Lastly, snRNA-seq analysis recapitulates transcriptional patterns associated with gene expression changes during spermatogenesis between ST and SR testes. Our results suggest the two candidate genes' significant role involving in the drive in this species, providing evidence that rapid evolution of genes disrupting spermatogenesis is important source of intragenomic conflict.

119 The genetic basis of cardiac glycoside resistance in wild-caught Drosophila melanogaster *Arya Rao*, Peter Andolfatto Columbia University

A significant number of unrelated herbivorous insects have repeatedly and independently evolved the ability to feed on plants that produce toxic secondary compounds called cardiac glycosides (CGs). CGs inhibit the function of Na+,K+-ATPase (NKA), a medically important enzyme in animals that is necessary for many processes including neural function and muscle contraction. Previous work identified a small number of adaptive amino acid substitutions in the alpha-subunit (ATPα) of NKA that confer the enzyme with resistance to CG inhibition. Further, engineering the native ATPα of D. melanogaster to carry one or more of these adaptive substitutions results in flies that are substantially resistant to CG toxicity. We show that wild-collected D. melanogaster strains harbor substantial genetic variation in sensitivity to CG-toxicity. By conducting GWAS on 180 Drosophila melanogaster Genome Reference Panel lines, we show that this CG resistance does not map to ATPα, but maps to a number of genes implicated in maintaining physiological barriers to solute diffusion and cellular and behavioral stress response. This information can be used to predict additional targets of recurrent adaptation in diverse CG-tolerant taxa. In addition, these results yield insight into the development of drugs to treat a number of Na+,K+-ATPase-associated neurological and physiological disorders in humans.

120 Old Hormones, new tricks: Juvenile Hormones ensure primordial germ cells reach the embryonic somatic gonad *Lacy Barton*[1], Justina Sanny[1], Emily P Dawson[1], Rebecca Spokony[2], Ruth Lehmann[3] 1) New York University, Skirball Institute; 2) Baruch College, CUNY; 3) The Whitehead Institute, MIT

Proper germ cell development is crucial for the health and fertility of subsequent generations. Emerging evidence suggests that secreted isoprenoids, such as retinoic acids (RAs) in vertebrates and juvenile hormones (JHs) in insects, impact several aspects of fertility. However, the mechanisms by which these potent molecules support reproductive development remain unclear. Here, we sought to elucidate the functions of JHs in primordial germ cell development. We made a GFP-based JH reporter, which revealed that JH signaling is first active in the *Drosophila* embryonic mesoderm as primordial germ cells emerge from the endoderm and begin to migrate within the mesoderm toward the somatic gonad. Consistent with this germ cell-proximal signaling pattern and previous work (Niwa, *et al*., 2008), we find that expression of a gene encoding the key JH biosynthesis enzyme, *jhamt*, is enriched near germ cells as they migrate toward the gonad and that JH degradation enzymes are expressed in areas that germ cells migrate away from. By generating several new double and triple mutant animals, we find that both JH biosynthesis and degradation enzymes are required for germ cells to efficiently colonize the developing somatic gonad. Together, these data suggest that JHs, which act systemically in larval and adult animals, act locally in the *Drosophila* embryo and that local dynamics in JH bioavailability may be needed to facilitate germ cell migration. To isolate autonomous vs non-autonomous requirements, we turned to an *in vitro* migration assay using primordial germ cells isolated by FACS, finding that pure JH III or the JH mimic, methoprene, is sufficient for germ cell migration *in vitro*. Interestingly, results from several experiments suggest that this newly uncovered function for JHs does not involve classical, nuclear receptor-mediated transcription, but rather calcium-linked cytosolic factor, phospholipase C. Collectively, these findings add to the growing appreciation of the diverse roles of secreted steroid and isoprenoids throughout the germline lifecycle in species that span both invertebrates and vertebrates. In mice, enzymes that synthesize RAs are also expressed in the developing somatic gonad as germ cell colonize this tissue. We find that, like JH in *Drosophila*, RA is sufficient for mouse germ cell migration *in vitro,* leaving open the possibility that this newly uncovered role in reproductive development may be conserved.

121 The role of *Drosophila* germ granules in regulating mRNA stability during germ cell development *Anna Hakes*, Elizabeth Gavis Princeton University

Germ granules, membraneless organelles containing mRNAs and proteins required for germline development, are a characteristic feature of germ cells. In *Drosophila,* germ granules form at the posterior of the oocyte and are subsequently segregated to the germ cell progenitors, called the pole cells, which bud from the posterior of the embryo. After pole cell formation, the germ granules increase in size and persist at least until the pole cells coalesce in the gonad. The persistence and evolutionary conservation of germ granules suggest that they play an important role in RNA regulation during germ cell development, but this role is not fully understood. We found that components of the mRNA decay pathway are sequentially recruited to the germ granules as these granules begin to increase in size. This association coincides with a dramatic decrease in the levels of two granule mRNAs, *nos* and *pgc*. By contrast, another germ granule mRNA, *cycB*, is protected from this degradation throughout embryogenesis. Our findings thus suggest that after pole cell formation, the germ granules play a dual role, selectively protecting some mRNAs while promoting

the degradation of others, which is distinct from their protective role prior to pole cell formation. Additionally, we have found that the recruitment of mRNA decay proteins to the germ cells is dependent on translation. Together, these data indicate that there is a temporally regulated change in germ granule function in the pole cells, suggesting that the germ granules evolve throughout development to meet the needs of the germ cells. Experiments are in progress to determine what factor is translationally activated to trigger this shift in germ granule function. Since germ granules are one of many types of RNA-rich membraneless organelles, understanding their function in regulating RNA stability can provide general insights into the roles of membraneless organelles in post-transcriptional regulation.

122 A feedback loop between heterochromatin and the nucleopore complex controls germ-cell to oocyte transition during Drosophila oogenesis *Kahini Sarkar*[1], Noor M Kotb[2], Alex Lemus[1], Eliott T Martin[1], Alicia McCarthy[3], Justin Camacho[1], Ayman Iqbal[1], Alex M Valm[1], Morgan A Sammons[1], Prashanth Rangan[1] 1) Department of Biological Sciences/RNA Institute, University at Albany SUNY, Albany, NY ; 2) Department of Biomedical Sciences, The School of Public Health, University at Albany SUNY, Albany, NY ; 3) Current address: 10x Genomics Headquarters, 6230 Stoneridge Mall Rd, Pleasanton, CA

Germ cells differentiate into oocytes that become totipotent upon fertilization. How the highly specialized oocyte acquires this distinct cell fate is poorly understood. During *Drosophila* oogenesis, H3K9me3 histone methyltransferase SETDB1 translocates from the cytoplasm to the nucleus of germ cells concurrent with oocyte specification. Here, we discovered that nuclear SETDB1 is required to silence a cohort of differentiation-promoting genes by mediating their heterochromatinization. Intriguingly, SETDB1 is also required for the upregulation of 18 of the ~30 nucleoporins (Nups) that comprise the nucleopore complex (NPC). NPCs in turn anchor SETDB1-dependent heterochromatin at the nuclear periphery to maintain H3K9me3 and gene silencing in the egg chambers. Aberrant gene expression due to loss of SETDB1 or Nups results in loss of oocyte identity, cell death and sterility. Thus, a feedback loop between heterochromatin and NPCs promotes transcriptional reprogramming at the onset of oocyte specification that is critical to establish oocyte identity.

123 Single-cell testes expression of ampliconic meiotic drivers on the sex chromsomes of *Drosophila miranda* *Kevin Wei*, Kamalakar Chatla, Doris Bachtrog University of California Berkeley

Sex-ratio meiotic drivers act during spermatogenesis to cause unequal production of X- and Y-bearing sperm. The presence of a driver on one chromosome reduces the fitness of the other and puts selection pressure for a suppressor to emerge counteracting the sex-ratio imbalance. This conflicting dynamic of X- and Y-linked drivers and suppressors is thought to underlie copy number amplifications of meiotic genes on sex chromosomes which has been observed all across animals. The recently formed sex chromosomes of *Drosophila miranda* is an extreme case; on both the neo-X and neo-Y chromosomes, hundreds of previously autosomal genes with meiotic function have massively amplified within ~1.5 million years. This system provides the opportunity to elucidate how these gene amplicons manipulate spermatogenesis to confer transmission advantage. To this end, we applied single-cell RNA-seq on *D. miranda* testes to understand their spatiotemporal regulation. 10,095 cells were recovered and placed into 14 clusters corresponding to different testis cell types including sperm cells at varying stages of spermatogenesis. Of the 128 multi-copy gene families, the majority are most highly expressed in either the spermatocytes (n = 74), where meiosis occurs, or the premeiotic spermatogonia (n = 28). Interestingly, the expression of ampliconic X-linked genes, on average, peaks in early spermatocytes, while ampliconic Y-linked genes lags behind, peaking in late spermatocytes and lasting through sperm individualization. Because X-linked drivers can be suppressed via complementary small RNAs, we further generated D. miranda testes small RNA-seq and found abundant production of 21bp small RNAs from the ampliconic genes strongly implicating down regulation via the small interfering RNA silencing pathway. Moreover, genes that are co-amplified on both sex chromosmes and have pre-meiotic expression show disproportionally high siRNA production from the Y-linked ampliconic gametologs. These results suggest that Y-linked ampliconic genes may act to downregulate their X-linked counterparts, and maintenance of equal sex ratio likely depends on the premeiotic dosage balanace of X-linked expression and Y-linked siRNA production.

124 Ecdysone signaling times border cell migration by regulating protrusive activity and cell-cell adhesion *Mallika Bhattacharya*, Michelle Starz-Gaiano University of Maryland, Baltimore County

Migratory cells play a significant role in spatiotemporally-regulated physiological processes such as normal embryonic development and wound healing. Dysregulation of these cells has severe implications in birth defects and diseases such as cancer. Border cell migration, during *Drosophila* oogenesis, serves as a useful model to study collective cell migration *in vivo.* Derived from the anterior epithelium of the egg chamber, the border cell cluster migrates toward the posteriorly-located oocyte in a temporally regulated manner. Developmental timing in flies is largely controlled by the sole steroid hormone ecdysone, which binds to a heterodimeric receptor complex that regulates transcription. Our work focuses on understanding how signaling by the ecdysone receptor (EcR), a conserved nuclear hormone receptor, affects border cell migration. Downregulation of ecdysone signaling results in delayed migration as a result of late detachment from the epithelium and slower migration. The directional translocation of the cluster, in response to chemoattractants,

requires the extension of actin-rich protrusions that adhere to other cells in the migratory path. Live imaging video analysis revealed that clusters expressing transcriptionally-inactive EcR extend fewer functional protrusions. Protrusion stabilization, cluster integrity, and migration are contingent on optimal distribution of the adhesion molecule, E-cadherin. Immunofluorescence characterization of E-cadherin showed variations in the levels and localization in border cells with reduced ecdysone signaling. We hypothesize transcription by EcR is important for migration and could explain some of the above phenotypes. Thus, we are studying potential migratory roles of EcR targets. Additionally, we are assaying EcR binding sites within migration-specific target gene loci using bioinformatic and chromatin immunoprecipitation analyses. Interestingly, an EcR transcriptional reporter is specifically activated in the cluster despite the apparent availability of the signalling components throughout the egg chamber. We are investigating possible reasons for transcriptional specificity such as genetic interactions between chromatin regulators and EcR. Elucidating the role of EcR in cell migration kinetics will be a useful guide to improve our understanding of nuclear hormone receptors in development and disease.

125 Cell intruder targeting system mediates paternal mitochondrial destruction after fertilization in *Drosophila* *Sharon Ben-Hur*, Sara Afar, Eli Arama Weizmann institute of science, Israel

Uniparental inheritance of mitochondria is one of the most conserved phenomena across evolution, occurring in diverse species, ranging from fungi and plants to mammals. In almost all animals, the sperm enters the egg with its mitochondria, resulting in a short period where mitochondria from both parents reside within the forming zygote. However, rapidly after fertilization, sperm mitochondria are eliminated, leaving the zygote solely with maternal mitochondria, which will further propagate in the developing embryo. Why and how paternal mitochondria are eliminated remain a matter of speculation and controversy, respectively. It is believed that massive heteroplasmy (i.e. the presence of more than one mitochondrial (mt)DNA type in a cell) is deleterious to the organism, but the overwhelming number of egg mitochondria (up to 600,000 in human) as compared with sperm mitochondria (50-75 in human) could not lead to massive heteroplasmy. Furthermore, in some organisms, such as *Drosophila*, sperm mtDNA is eliminated already during spermatogenesis, yet sperm vacuolar mitochondria are still targeted for destruction after fertilization. Whether the mechanism of paternal mitochondria elimination is conserved across species is unclear. Some studies suggest a passive mechanism whereby paternal mitochondria are diluted beyond detection by the overwhelming number of maternal mitochondria. On the other hand, other studies, including in *Drosophila*, suggest an active egg-derived paternal mitochondrial destruction mechanism.
Here, I will present our recent unpublished discovery of a non-canonical cell intruder targeting system, consisting of combined elements from the endocytic, phagocytic, and autophagic systems, which specifically targets sperm mitochondria for destruction after fertilization in *Drosophila*. These elements are conserved between *Drosophila* and human, implying that similar mechanisms might also operate to eliminate paternal mitochondria, at least in all flagellated sperm organisms.

126 Transcriptome analysis implicates circadian clock genes in Sex Peptide-dependent post-mating responses in *Drosophila melanogaster* females *Sofie Delbare*, Sara Venkatramen, Martin Wells, Sumanta Basu, Mariana Wolfner, Andrew Clark Cornell University

Sex Peptide (SP), a seminal fluid protein of *D. melanogaster* males, elicits an array of post-mating responses in females, including increased egg laying, activity and food intake, with a preference for proteins and a switch from carbohydrate to amino acid metabolism. To determine how one protein can have such widespread effects, we set out to dissect the genetic architecture of the female's response to SP, and determine whether SP alters the expression of several regulators targeted to specific post-mating responses or acts on a pleiotropic regulator that controls multiple responses. We performed bulk RNA-seq of female heads at 10 time points within the first 24 hours after mating, sampling virgin females, females mated to control males and females mated to SP-null males. Using gene- and exon-level differential expression (DE) analyses, we find 666 genes and 86 exons that are DE across the 3 treatments. The earliest DE features include 3 regulators of circadian rhythms (*cwo*, *Clk*, *Pdp1*), leading us to hypothesize that SP acts on the circadian clock to induce at least a subset of post-mating responses. Consistent with this, we find that 16% of the 752 DE features have circadian expression patterns in virgin females, and their rhythms are altered after mating. Cluster analysis shows that features with altered circadian rhythms follow either of two patterns: upregulated during both day and night, or downregulated specifically during the night, relative to virgin females and females that did not receive SP. The downregulated genes include ones involved in glycogen biosynthesis, transcription factors involved in energy metabolism, and *Acer*, whose downregulation is known to cause increased activity at night. Upregulated genes include ones involved in amino acid metabolism, transcription factors including *Clk*, and signaling molecules. Among these are exons of *foraging,* a well-studied gene with roles in feeding behavior, of which some exons follow a circadian rhythm in virgin females, but no longer after receipt of SP. Overall, our transcriptome analysis suggests that SP might act on genes in the circadian clock to alter the expression of target genes with various functions. We are further investigating networks of genes and exons using an empirical Bayes clustering approach and transcription factor motif enrichment analyses, to better understand how SP orchestrates multiple post-mating responses.

127 Genetic coordination of sperm morphology and seminal fluid proteins promotes male reproductive success

in *Drosophila melanogaster* *Jake Galvin*[1], Erica Larson[2], Sevan Yedigarian[1], Mohammad Rahman[1], Kirill Borziak[3], Michael DeNieu[2], Mollie Manier[1] 1) George Washington University, Washington DC; 2) University of Denver, Denver CO; 3) Syracuse University, Syracuse NY

Spermatozoal morphology is highly variable both among and within species and in ways that can significantly impact fertilization success. In *Drosophila melanogaster*, paternity success depends on sperm length of both competing males and length of the female's primary sperm storage organ. We found that genes upregulated in long sperm testes are enriched for lncRNAs and seminal fluid proteins (Sfps). Transferred in seminal fluid to the female during mating, Sfps are secreted by the male accessory glands (AG) and affect female remating rate, physiology, and behavior with concomitant advantages for male reproductive success. Despite being upregulated in long sperm testes, they have no known function in testis tissue. We found that Sex Peptide and ovulin (Acp26Aa) knockouts resulted in shorter sperm, suggesting that Sfps may regulate sperm length during spermatogenesis. However, knockout of AG function did not affect sperm length, suggesting that AG expression has no influence on spermatogenic processes. We also found that long sperm males are better able to delay female remating, suggesting higher Sfp expression in AG. These results might suggest that long sperm males have a double advantage in sperm competition by both delaying female remating, likely through transfer of more Sfps, and by resisting sperm displacement. However, we also found that this extra advantage does not necessarily translate to more progeny or higher paternity success. Thus, we found that multiple components of the ejaculate coordinate to promote male reproductive success at different stages of reproduction, but the realized fitness advantages in sperm competition are uncertain.

136 Visceral organ morphogenesis via calcium-patterned muscle constrictions *Noah Mitchell*[1], Dillon Cislo[1], Suraj Shankar[1,2], Yuzheng Lin[1], Boris Shraiman[1], Sebastian Streichan[1] 1) University of California Santa Barbara, Santa Barbara, CA; 2) Harvard University, Cambridge, MA

During development, interacting tissue layers orchestrate complex shape changes to form visceral organs. Tracing the dynamics and mechanical interactions across tissue layers and across scales -- from cell to tissue, to entire organs -- remains an outstanding challenge. Here, we study the embryonic *Drosophila* midgut as a model visceral organ. Using light-sheet microscopy, genetics, computer vision, and tissue cartography, we reconstruct in toto the dynamics of individual tissue layers to map the time course of shape across scales. We identify the kinematic mechanism driving shape change by linking out-of-plane motion to active contraction patterns, revealing a convergent extension process in which cells deform as they flow into deepening folds. Optogenetic perturbations of muscle contractility reveal that muscle activity drives these contraction patterns and induces cell shape changes in the adjacent endoderm layer. This induction cascade relies on calcium pulses in the muscle, under the control of hox genes. Our study of multi-layer organogenesis reveals how genetic patterning in one layer triggers a dynamic molecular mechanism to control a physical process in the adjacent layer, orchestrating whole-organ shape change.

137 Maintaining symmetry in morphogenetic movements *Celia Smits*[1,2], Sayantan Dutta[1,3], Sebastian Streichan[4,5], Stanislav Shvartsman[1,2,3,6] 1) Lewis Sigler Institute for Integrative Genomics, Princeton University, NJ; 2) Department of Molecular Biology, Princeton University, NJ; 3) Department of Chemical and Biological Engineering, Princeton University, NJ; 4) Department of Physics, University of California Santa Barbara, CA; 5) Biomolecular Science and Engineering, University of California Santa Barbara, CA; 6) Center for Computational Biology, Flatiron Institute, Simons Foundation, NY

Morphogenetic processes must resist biological noise and environmental perturbations to faithfully construct an adult organism. We are investigating the mechanisms underlying such robustness in *Drosophila* embryogenesis. Here, we focus on bilateral symmetry maintenance during body axis extension. In *Drosophila*, the body axis extends parallel to the long axis of the embryo during gastrulation. However, we present evidence that there is a certain amount of left-right variation in midline position that is corrected as the tissue elongates. To determine the origin of this correction, we investigated a class of mutants where embryos exhibit a characteristic "corkscrew" phenotype. We find that in these mutants, the midline begins twisting along the left-right axis shortly after the onset of axis extension. Using a combination of classical genetics, light sheet microscopy, particle image velocimetry, dimensionality reduction and computation modelling, we characterize the mechanics and genetics that underlie this twisting. Finally, we propose a model where invagination of the posterior midgut acts to disperse the forces of convergent extension in a bilaterally symmetric manner, which is required to maintain bilateral symmetry in the face of stochastic differences in lateral extension rates. Overall, this work indicates that coordination of morphogenetic movements and mechanics act to rein in random biological noise and ensure correct tissue flow and proper morphogenesis.

138 Malvolio, a Fork head target metal ion transporter, is required for salivary gland morphogenesis *Srihitha Akula*, Rajprasad Loganathan, Tony Zou , Rika Maruyama, Deborah Andrew 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). The family members (NRAMP1 and NRAMP2) function as general metal ion transporters that use proton motive force to transport Fe^{2+}, Cu^{2+}, Mn^{2+}, Zn^{2+}, Cd^{2+}, Ni^{2+}, and

Co^{2+}. The key known roles for NRAMPs are in maintaining ion homeostasis essential to support the antimicrobial activities of phagocytic cells. Our interest in *Mvl* stems from its high-level Fkh-dependent expression in the embryonic salivary gland (SG), an ideal model system for studying tissue morphogenesis and functional specialization. To begin to uncover the role of *Mvl* in the SG and in other tissues, 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 characterization of *Mvl* loss-of-function revealed that although zygotic loss of *Mvl* does not affect viability, development is delayed, with *Mvl^{exc1}/Df(Mvl)* adults eclosing 48 – 72 hours after their balancer-containing siblings. To determine if loss of *Mvl* affects iron accumulation and/or storage, we examined *Mvl^{exc1}* homozygous larvae and found that the Iron-storing epithelial cells of the anterior midgut lose their Ferritin enrichment, whereas the brain Ferritin levels were unaffected (based on Ferritin-GFP signal intensity). Whereas zygotic loss of *Mvl* had only mild effects on SG morphology and larval cuticles, the combined maternal and zygotic loss of *Mvl* resulted in earlier, conspicuous defects in SG morphology as well as severe defects in the larval cuticle, with a near complete loss of ventral denticles and dorsal hairs. *Mvl* homozygotes also featured a loose assemblage of an unidentified CrebA+ population of cells in the anterior region of the embryo. The examination of junctional proteins in *Mvl* null embryos revealed that levels and localization of the adherens junction protein E-cadherin and polarity marker Bazooka were comparable in *Mvl* and wild-type SGs, whereas the levels of the apical polarity determinant Crumbs were notably reduced. 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 and to both early and late endosomes. Based on these findings, we hypothesize that Mvl may have a role in the trafficking of either newly synthesized or recycled Crb to the sub-apical domain.

139 Defining the structure and function of the multivalent protein network at cell-cell adherens junctions during morphogenesis *Anja Schmidt*[1], Tara Finegan[2], Kristi Yow[1], Mark Peifer[1] 1) University of North Carolina at Chapel Hill, NC; 2) Syracuse University, NY

Adherens junctions play a major role in assuring cell-cell adhesion, and through their linkage to the cytoskeleton, mediate dramatic shape changes and rearrangements during embryonic morphogenesis. The junctions need to be strong yet also dynamic, to allow cell rearrangement without tissue rupture. We want to understand how junction-associated proteins, like the multi-domain scaffold Polychaetoid (Pyd; fly homolog of ZO-1), work together to allow junctions to react dynamically to cell shape change and force generation. We hypothesize that the junctional complex and its linkage to the cytoskeleton involves multivalent connections among multiple junction-associated proteins, assembled into an underlying structured molecular complex that mediates binding to the cytoskeleton. I am using structured illumination microscopy (SIM) to explore the junctional localization of these proteins in high resolution. My preliminary data suggest that the core junction-associated proteins Canoe (Cno) and Armadillo (β-Catenin) localize to alternating clusters along the zonula adherens of cell-cell borders, often with little overlap. Furthermore, I found that Pyd surrounds these core junctional proteins in a cloud-like pattern of puncta. I am now broadening my analysis to include other junction-associated proteins like Bazooka (Par-3), and Non-muscle myosin II. Next, I will analyze how these patterns change in *pyd* mutants. In parallel, I am exploring the functional role of Pyd as one player in this robust network. While Pyd is not absolutely essential for embryonic viability, mutants display defects in cell rearrangements during germband extension. Mutants have stacks of elongated cells, an elevated number of rosettes and junctional disruptions at tricellular junctions and anterior-posterior cell borders, suggesting defects in responding to elevated force on cell-cell junctions. However, these occur without major tissue rupture, suggesting other proteins act in parallel. To explore a hypothesized change in junction dynamics in *pyd* embryos, I am applying live imaging during germband elongation. Furthermore, we are now expanding our analysis to include other players in this protein network, including the tricellular junction protein Sidekick, and Canoe's regulators Rap1 and the GEF Dizzy.

140 A feedback mechanism mediated by actomyosin activity-dependent apical targeting of Rab11 vesicles reinforces apical constriction *Wei Chen*, Bing He Dartmouth College

During tissue morphogenesis, cell shape changes resulting from cell-generated forces often require active regulation of intracellular trafficking. How mechanical stimuli influence intracellular trafficking and how such regulation impacts tissue mechanics are not fully understood. Non-muscle myosin II (Myosin II) is an important force generator in many cell and tissue types that acts through its contraction on actin network. In this study, we identify an actomyosin activity-dependent mechanism involving Rab11-mediated trafficking in regulating apical constriction in the *Drosophila* embryo. During *Drosophila* mesoderm invagination, apical actin and Myosin II (actomyosin) contractility induces accumulation of Rab11-marked vesicle-like structures ("Rab11 vesicles") near the apical membrane. These Rab11 vesicles are not derived from apical endocytosis. Instead, apical accumulation of Rab11 vesicles is associated with constant vesicle transport along cell apical-basal axis, which is mediated by microtubule motor dynein. Interestingly, actomyosin contractility promotes apical targeting of Rab11 vesicle by inhibiting the reverse transport, the resulting directional bias towards apical side therefore contributes to the apical accumulation of Rab11 vesicles. At the apical domain, Rab11 vesicles are targeted to the adherens junctions (AJs). The apical accumulation of Rab11 vesicles is essential to prevent fragmented apical AJs, breaks in the supracellular actomyosin network and therefore promotes the stepwise, ratchet-like cell area

reduction and overall apical constriction. This function of Rab11 is separate from its earlier role in promoting apical Myosin II accumulation. These findings suggest a feedback mechanism between actomyosin-mediated apical constriction and Rab11-mediated intracellular trafficking that regulates the force generation machinery during tissue folding.

141 Mechanical cues planar polarize Pins and orient divisions during *Drosophila* gastrulation *Jaclyn Camuglia*[1], Soline Chanet[2], Adam Martin[1] 1) Massachusetts Institute of Technology; 2) Collège de France

Epithelial morphogenesis and homeostasis are properly achieved by oriented cell divisions that occur along specific directions relative to both the epithelial plane and the polarity of an organ/organism. In epithelial sheets of cells, divisions can occur either perpendicular or parallel (planar) to epithelial sheets. Planar cell divisions can further exhibit polarity in specific orientations along epithelial tissues (planar polarization). Spindle orientation is often achieved by a complex of Pins/LGN, Mud/NuMa, Gai, and Dynein, which interacts with astral microtubules to rotate the spindle. Here, we identify the mitotic domains of the early embryo as a system to study Pins-mediated planar polarized divisions during morphogenesis. Mitotic domains (MDs), 1, 3, 5, and 14 exhibit oriented division and these oriented divisions are dependent on proper Pins localization and activity. We find that the divisions within these domains are oriented within 30 degrees of the anterior-posterior (AP) axis of the embryo and that Pins is localized in planar polarized crescents within the domains. Disruption of Pins localization and activity via expression of a myristoylated version of Pins leads to misoriented divisions. It is currently unknown what causes Pins localization in this context. Both planar polarity proteins and mechanical force have been shown to localize Pins and cue spindle orientation during division. We find that in MDs 1, 3, 5, and 14 canonical planar cell polarity pathways are not solely responsible for the orientation of the divisions. Further, we find that adherens junctions, which mechanically couple cells within epithelia, are necessary for the orientation of the divisions. Disruption of a-catenin disrupts division orientation and pins polarization. Our findings suggest mechanical forces, specifically those generated during morphogenesis are responsible for Pins polarization and spindle orientation. Disruption of forces through a chemical inhibitor, laser ablation, and genetic perturbation causes divisions to fail to orient along the AP axis and abolishes Pins polarization. To our knowledge, demonstrating that mechanical force polarizes Pins to mediate division orientation has not yet been shown *in vivo*.

142 Tissue geometry reorients in-plane homeotic tension to promote folding. Aurélien Villedieu[1,2], Lale Alpar[1,2], Isabelle Gaugue[1,2], Amina Joudat[1,2], François Graner[3], *Floris Bosveld*[1,2], Yohanns Bellaïche[1,2] 1) Institut Curie, PSL Research University, CNRS UMR 3215, INSERM U934, Paris, France.; 2) Sorbonne Universités, UPMC Univ Paris 06, CNRS UMR 3215, INSERM U934, Paris, France.; 3) Matière et Systèmes Complexes, Université de Paris - Diderot, CNRS UMR 7057, Paris, France

Shape is a conspicuous and fundamental property of biological systems entailing the function of organs and tissues. While much emphasis has been put on how tissue tension and mechanical properties drive shape changes, how a given initial shape influences subsequent morphogenesis remains poorly characterized. Here, we analyzed invagination of the *Drosophila* neck epithelium, which exhibits a curved radial geometry prior to its formation. We found that neck folding depends on an in-plane tension promoted by the *Deformed* (*Dfd*) homeotic gene. By varying tissue curvature, we established that tissue geometry promotes an inward force driving folding. Accordingly, the interplay between Dfd-dependent in-plane tension and curvature explains the spatiotemporal dynamics of tissue invagination. Collectively, our work highlights how tissue geometry and homeotic regulation of tissue tension provide a simple design principle contributing to folding morphogenesis during development.

143 The flipside of tissue growth: how the two layers of the wing imaginal disc keep pace with each other *Sophia Friesen*, Iswar Hariharan University of California, Berkeley

Epithelial sheets of cells are a fundamental building block of animal tissue. To build functional, proportional organs, epithelia must coordinate their growth with the growth of other epithelial and non-epithelial tissues. How this synchronized growth occurs is a key question in developmental biology. To understand coordinated epithelial growth, we turned to the wing imaginal disc, a larval organ that develops into the adult wing and thorax. The wing disc consists of two layers of simple epithelia that are connected at their edges (similar to a pita pocket). These two layers have very different morphologies – the disc proper (DP) is composed of many tightly-packed columnar cells, while the peripodial epithelium (PE) is made up of relatively few cells that are very flat and broad – but despite their differences, the two layers grow at the same speed throughout larval development. We wanted to know what regulates growth of the PE, and how it keeps pace with the DP, in order to better understand how epithelial proportionality is maintained during development.
We found that even though the two layers grow at the same speed, they use very different pathways to do so. Growth of the DP absolutely requires the secreted morphogens Hedgehog and Dpp, but we found that most PE growth can occur without these signals. In contrast, several components of the mechanosensitive Hippo pathway, including Yorkie (Yki) and multiple transcriptional cofactors, are critical for growth of the PE. Yki is required for PE survival and proliferation, as it is in many tissues, but it also mediates the dramatic cell shape changes that are unique to this layer of the disc.
Yki activity can be upregulated by mechanical stretching forces, and so could directly link growth of the PE to growth

of the DP. We speculate that DP growth stretches the PE, which increases PE Yki activity. Increased Yki activity, in turn, promotes growth of the PE, so that the two layers grow at the same speed. In this case, use of two very different growth paradigms – one based on morphogen gradients, and one based on mechanical force – seems to be the key to synchronized epithelial growth.

144 Uncovering how the pioneer transcription factor Grainy head binds and opens chromatin *Meghan Freund*, Andrew Rashoff, Tyler Gibson, Peter Lewis, Melissa Harrison University of Wisconsin-Madison

Multicellular organisms are made up of numerous differentiated cell types that perform unique functions. Beginning from a single cell, organisms undergo a journey of differentiation, resulting in the formation of this 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 structure can act as a barrier to transcription-factor binding. Specialized transcription factors, known as pioneer transcription factors, can overcome this barrier by binding to condensed chromatin and increasing chromatin accessibility at previously inaccessible loci. While pioneer factors share the ability to bind condensed chromatin, the exact mechanisms are not understood as these factors all act through divergent DNA-binding domains. Grainy head (GRH) is an essential pioneer factor that drives epithelial cell fate and when misexpressed, can lead to cancer. It is conserved across species ranging from worms to humans 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. Furthermore, while mammals have three GRH-like proteins, *Drosophila* encode GRH from a single gene, simplifying functional studies. To understand the properties of GRH that allow it to access compacted chromatin, we used in vitro studies to show that GRH can bind DNA in the context of nucleosomes. Using structural analysis, we identified mutations in the DNA-binding domain that mediate either sequence-specific or non-specific interactions and confirmed these properties *in vitro*. We are using these mutants to determine how this conserved protein interacts with DNA through its DNA-binding domain. We have further developed a tissue-culture system to investigate these mechanisms *in vivo*. Together our data will determine how GRH scans the genome and recognizes its motifs within inaccessible chromatin.

145 Image-based investigation of enhancer-promoter bridging in the *Drosophila* genome *Aleena Patel*[1], Leslie Mateo [1,2], Sedona Murphy[1], Angela Pogson[1], Alistair Boettiger[1] 1) Stanford University, Stanford, CA; 2) Genentech, South San Francisco, CA

In metazoans, cis-regulatory DNA sequences (enhancers) often lie kilobases away from the target genes' proximal transcription start sites (promoters), where transcriptional machinery is assembled. Various assays of the physical arrangement of sequences in 3D space have not yet solved controversial debates about which mechanism, or combination of mechanisms mediate such long-range communication in *Drosophila*. In mammals, ATP-dependent motors on the cohesin complex walking in opposite directions along DNA while tethered to one another may bridge distally located enhancers and promoters. This mechanism requires a DNA-bound factor such as the CCCTC binding factor (CTCF) to stall cohesin at the right loci. Despite a non-conserved function for CTCF in *Drosophila*, perturbations of cohesin components and regulators quantitatively change gene expression and suggest a possible role for active loop extrusion in enhancer-promoter contact. Alternatively, point-to-point loops could be formed by protein tethers that interact when thermal fluctuations in the DNA polymer bring their binding sites together. Several *Drosophila* DNA-binding factors are found near the boundaries of topologically associating domains (TADs) and may mediate physical tethering of select DNA loci by interacting or phase separating with one another.

We investigate these proposed mechanisms for *Drosophila* genome folding with optical reconstruction of chromatin architecture (ORCA), a super-resolution imaging technique. We have fluorescently labeled 80 5-kilobase regions spanning TADs that harbor enhancer-promoter loops for the genes *scyl* and *chrb* in *Drosophila* tissues. From the imaging data, we have reconstructed individual polymers tracing the DNA path in single cells. ORCA has been performed on embryos depleted of loop-associated factors, including a candidate tether, GAGA binding factor (GAF), the cohesin component, Rad21, the cohesin loader, NipB, and the cohesin unloader, WAPL. We find that GAF depletion specifically disrupts enhancer-promoter loop formation. Positive and negative perturbations of cohesin function have opposing effects on overall TAD compaction without disrupting the GAF-dependent loops. I will discuss how these results, and the insights of the *Drosophila* model system, are promising to expand current models of genome organization.

146 *Drosophila* genome architectural proteins form *in vivo* liquid -liquid phase separating *Bright Amankwaa*, Ryan Simmons, Ran An, Mariano Labrador University of Tennessee, Knoxville

Drosophila insulator proteins, which share genome architectural properties with their vertebrate counterparts, coalesce to form nuclear foci known as insulator bodies in response to osmotic stress. However, the mechanism behind the formation of these bodies remains unknown. Here, we identify signatures of liquid-liquid phase separation (LLPS) in insulator bodies. Mounting evidence implicates LLPS in the formation of membraneless organelles utilized for various

biological functions, including genome structure and function. We show that insulator proteins have a high disorder tendency, assemble into insulator bodies in a scaffold-client dependent manner, have extensive fusion behavior, and show sensitivity to 1,6-hexanediol. Further, we characterize the Cohesin subunits Rad21 and Wapl as well as the phosphorylated version of the histone variant H2Av (γH2Av) as novel components of insulator body condensates. Our data suggest a concerted role of Cohesin, γH2Av and insulator proteins in the formation of insulator bodies and under physiological conditions. We propose a model whereby these architectural proteins modulate the 3D genome organization of *Drosophila* through LLPS.

147 Chromatin state transitions in the *Drosophila* intestinal lineage gives new insights into cell type specification *Manon Josserand*[1], Natalia Rubanova[1], Owen Marshall[2], Louis Gervais[1], Allison Bardin[1] 1) Institut Curie, PSL Research University, CNRS UMR 3215, INSERM U934, Stem Cells and Tissue Homeostasis Group, Paris, France; 2) Menzies Institute for Medical Research, University of Tasmania, Australia

Adult stem cells self-renew and differentiate into one or several cell types, thus ensuring tissue homeostasis. Understanding their regulation is crucial to have a better comprehension of uncontrolled proliferation and altered differentiation mechanisms occurring during tumorigenesis and age-dependent functional decline of tissues. Here, we aim to better understand what chromatin states are associated with adult stem cell activity *in vivo* in a homeostatic tissue using the *Drosophila* adult intestine as a model. Our lab has previously provided evidence of roles of conserved chromatin factors in controlling intestinal stem cell (ISC) proliferation (Gervais et al, 2019), highlighting their importance in the regulation of the intestinal lineage. Here, we expanded on these studies to investigate chromatin state changes associated with stem cell differentiation at the genome-wide scale. By generating cell-type specific whole-genome binding maps of 5 chromatin proteins (RNA Pol II, Brahma, Polycomb, HP1 and H1) using Targeted DamID and performing subsequent Hidden Markov modelling to define chromatin states, we found that 7 major chromatin states exist in the ISC lineage. Examining these states at specific genes revealed dynamic changes within the lineage. First, ISC-specific transcription factors such as *esg*, *klu*, *Sox100B* become marked by the Blue (Polycomb-enriched) state in differentiated cells. In contrast, components of signaling pathways regulating ISC activity are found in distinct states depending on the differentiated cell type, suggesting that they are repressed in different ways. In addition, the key transcription regulators of lineage specification including *pros* and *pdm1* undergo a transition from the Blue state (Polycomb-enriched) to active states upon differentiation, suggesting a role for Polycomb group proteins in repressing these genes in ISCs. While these data suggest a regulatory function of Polycomb-marked chromatin for control of the transcriptional hierarchy within the ISC lineage, we find that genes involved in differentiated cell physiology are instead associated with Histone H1- enriched Black chromatin. Indeed, physiology and metabolic activity-related genes follow a transition from the Black state in ISCs to active states upon their activation, suggesting a previously uncharacterized mode of regulation of physiology-related genes. On-going work will highlight the biological relevance of these chromatin transitions by perturbing specific states and studying the subsequent transcriptional changes and effects on proliferation and differentiation in the intestinal lineage. Overall, the extensive characterization of chromatin state changes during differentiation will provide a valuable resource to better understand the regulatory programs that control cell fate and identity, as well as physiological functions in this homeostatic tissue.

148 Condensin II loss ameliorates long-range chromosomal interactions in both active and inactive physical compartments within a chromosome territory *Randi Isenhart*[1], Son Nguyen[1], Leah Rosin[1], Olivia Crocker[1], Yemin Lan[2], Eric Joyce[1] 1) Department of Genetics, Perelman School of Medicine, University of Pennsylvania; 2) Department of Cell and Developmental Biology, Perelman School of Medicine, University of Pennsylvania

Metazoan genomes are folded into structures at a variety of length-scales, from chromatin loops and domains to higher-order interactions that connect distal genomic regions to form chromosome territories. Polymer modeling of Hi-C data suggests that chromosome folding is driven by a loop extrusion mechanism to both fold entire chromosomes into territories and restrict their interactions with other chromosomes. Recent work from our lab and others suggest that the condensin II loop-extruding complex specifically regulates chromosome territory formation and levels of inter-chromosomal interactions during interphase in *Drosophila* and human cells. To dissect the mechanism by which condensin II modulates genome folding, we are using a combination of Oligopaint FISH and Hi-C in *Drosophila* cultured cells to show that condensin II is required for proper inter-domain interactions in a chromatin state-nonspecific manner without altering interactions within domains. Optimized ChIP-Seq of a condensin II component reveals a novel class of genomic binding sites that are associated with long chromatin loops and colocalize with the gypsy complex, a strong insulator at the interface of active and inactive chromatin. *In vivo* data suggest that these condensin II-dependent interactions are important for proper silencing of heterochromatic sequences that are distal, but not proximal, to the pericentromeric heterochromatin. Overall, we propose a model in which condensin II extrudes chromatin into large loops until blocked by an insulator prior to cohesin activity early in interphase, thereby bridging distal interactions that fold chromosomes into distinct territories. Ongoing experiments will determine the functional consequences of condensin II loss on gene regulation.

149 Histone gene replacement reveals functional independence, redundancy and synergism between lysine 36 of

H3.2 and H3.3 *Harmony Salzler*[1], Vasudha Vandadi[1], Sally Boerma[2], John Brown[1], A Gregory Matera[1] 1) UNC Chapel Hill; 2) Carleton College

Polycomb silencing is an epigenetic mechanism for determining cell-type specific gene expression programs, ensuring that genes specifying alternative lineages remain repressed. The best-studied examples of this phenomenon are the *Drosophila* Hox genes, which specify the body plan. To ensure proper development of body segments, Hox genes must be expressed at certain points along the body axis and repressed at others.

One critical step in this process is trimethylation of histone H3 lysine 27 (H3K27me3) by the Polycomb repressive complex 2, PRC2. Importantly, PRC2 can also "read" the H3K27me3 mark, enabling cis-linked spreading of H3K27me3, and formation of repressive chromatin domains essential for proper Hox gene expression. To antagonize the spreading of H3K27me3 into other active genes, evidence suggests that cells utilize di- and tri-methylation of H3 lysine 36 (H3K36me2/3) to abut these repressive domains. A mechanism by which H3K36me2/3 halts spreading of facultative heterochromatin was unknown until recent structural studies revealed that unmethylated H3K36 occupies a crucial role in positioning H3K27 into the active site of its cognate methyltransferase, EZH2 (the mammalian ortholog of *Drosophila* enhancer of zeste E(z)). This finding suggests that H3K36me2/3 sterically hinders *E(z)* activity and that mutation of H3K36 would elicit strong Polycomb phenotypes. One puzzle that emerged from this work is that when Hox gene expression was examined *in vivo*, mutants of the replication-dependent histone H3 genes ($H3.2^{K36R}$ and $H3.2^{K36A}$) displayed relatively mild Polycomb phenotypes, despite inhibitory effects of these mutations on H3K27 trimethylation *in vitro*.

In addition to H3.2, metazoan genomes also contain the replication-independent histone variant, H3.3, which differs from H3.2 by only 4 amino acids. We hypothesized that H3.3 might function redundantly to enable Polycomb mediated gene repression in the H3.2 K36R mutants. Therefore, we generated an $H3.3^{K36R}$ mutant to directly compare Polycomb mediated gene silencing between $H3.3^{K36R}$, $H3.2^{K36R}$, and $H3.3^{K36R}H3.2^{K36R}$ combined mutant animals. Our studies in embryos, first instar larvae, and adults demonstrate that H3.2K36 and H3.3K36 cooperate to maintain H3K27 trimethylation and Hox gene silencing by different, but synergistic mechanisms.

150 Single-cell chromatin accessibility in *Drosophila melanogaster* human tauopathy model *Eve Lowenstein*[1], Andrew Adey[1], Doris Kretzschmar[2] 1) Department of Molecular & Medical Genetics, Oregon Health & Science University, Portland, OR; 2) Center for Research on Occupational and Environmental Toxicology, Oregon Health & Science University, Portland, OR

Frontotemporal dementia (FTD) is a neurodegenerative disease associated with mutations in the microtubule binding protein Tau. The clinical presentation of FTD is heterogeneous with patients exhibiting parkinsonism, dementia, atrophy in the temporal lobes, and personality changes. Current treatments can mitigate aspects of the behavioral changes associated with FTD, however, no therapies are available to slow the progression. Since patient sample procurement is restricted to post-mortem tissue, our understanding of the progression and underlying pathogenic mechanisms of this disease is limited. Recent work in model systems and post-mortem tissue has shown that expression of FTD-associated mutant Tau may lead to epigenetic modifications that alter gene expression. In our lab, we model FTD using *Drosophila*, which allows us to conduct longitudinal studies to observe FTD progression throughout the adult lifespan. The adult *Drosophila* expressing FTD-associated mutant human Tau (hTau) have age-dependent neurodegenerative vacuoles, axonal changes, locomotion defects and impaired memory while flies expressing normal hTau did not. This confirms that our models show pathogenic phenotypes associated with Tauopathies and it provides the basis to now use these models to identify molecular mechanisms of pathogenicity. We also found changes in a chromatin associated protein, Heterochromatin Protein 1 and hypothesized that FTD mutant Tau could alter chromatin accessibility. We used Single-cell Combinatorial Indexed Assay-for-Transposase-Accessible Chromatin using sequencing (sci-ATAC-seq) to assess how human Tau FTD mutations alter chromatin accessibility in the young and aged adult *Drosophila* brain for three clinically distinct Tau mutations (K369I, P301L, V337M). We generated >90,000 single-cell chromatin accessibility profiles from whole adult heads. Comparing our wildtype hTau insertion line to the FTD mutants revealed differentially accessible regions in both neuronal and glial cell populations. This work will further our understanding of chromatin structure in hTau knock-in models at single-cell resolution and provide insight into cell-type specific variation.

151 Simultaneous cellular and molecular phenotyping of embryonic mutants using single cell regulatory trajectories *Stefano Secchia*[1], Mattia Forneris[1], Tobias Heinen[2], Oliver Stegle[1,2], Eileen Furlong[1] 1) Genome Biology Unit, European Molecular Biology Laboratory (EMBL), Heidelberg, Germany; 2) Division of Computational Genomics and Systems Genetics, German Cancer Research Center (DKFZ), Heidelberg, Germany

Developmental progression and cellular diversity are largely driven by transcription factors (TFs), yet characterizing their loss-of-function phenotypes remains challenging and often disconnected from their underlying molecular mechanisms. To overcome this, we have combined single cell regulatory genomics with loss-of-function mutants to simultaneously assess both cellular and molecular phenotypes.

We performed single cell chromatin accessibility profiling by sci-ATAC-seq for over 20,000 cells at eight overlapping time-points during *Drosophila* mesoderm development, ensuring that key developmental transitions are captured and can be directly traced, including stages of multipotency, specification and terminal differentiation. This dataset captures the establishment of regulatory landscapes in the nascent embryonic mesoderm at high resolution and its progression along developmental time. We demonstrate that this information can reconstruct multiple developmental trajectories of all major and rare cell-types, including the heart, and uncover the enhancers and TFs involved.

To systematically assess the TFs mutant phenotypes, we developed a streamlined strategy to robustly profile chromatin accessibility and digitally genotype mutant cells coming from embryo collections of mixed genotypes. Applying this approach to four TF mutants could uncover and quantify their characterized phenotypes *de novo*, and discover new ones, while simultaneously revealing their regulatory input and mode of action. We observe strikingly different outcomes in cell fate, as one mutant (*Mef2*) results in abnormal 'new' cell states, while other TF mutants appear to block cell populations much earlier along their developmental trajectory.

Our approach is a general framework to dissect the functional input of TFs in a systematic unbiased manner, uncovering both cellular and molecular phenotypes at a scale and resolution that was not feasible before.

152 A KDM5-Prospero transcriptional axis functions during early neurodevelopment to regulate mushroom body formation *Hayden Hatch*[1], Helen Belalcazar[1], Owen Marshall[2], Julie Secombe[1] 1) Albert Einstein College of Medicine; 2) University of Tasmania

Intellectual disability (ID) disorders affect up to 2% of the population and are characterized by an IQ score less than 70 with deficits in adaptive functioning. Our research focuses on the KDM5 family of transcriptional regulators (KDM5A, KDM5B, KDM5C, and KDM5D), mutations in which account for 1-3% of inherited ID ranging from mild to severe. Although recent advances in comparative genomic hybridization and whole exome sequencing have revealed approximately 70 unique genetic variants in *KDM5C* segregating in families with inherited ID, the molecular mechanisms by which this family of proteins impacts neuronal function remain largely unknown, leaving patients without effective treatment strategies.

Here, we utilize the *Drosophila* Mushroom body (MB), a major learning and memory center within the fly brain, to demonstrate that *Drosophila* KDM5 is specifically required within ganglion mother cells (GMCs) and immature neurons for proper neurodevelopment. Utilizing Targeted DamID (TaDa), we identify a core network of KDM5-regulated genes within GMCs and immature neurons that are critical modulators of neurodevelopment. Significantly, we find that a majority of these genes are direct targets of Prospero (Pros), a transcription factor with well-established roles in neuronal growth and guidance. We further demonstrate that *pros* genetically interacts with *kdm5* to orchestrate a transcriptional program critical for proper MB development.

To better understand the molecular mechanisms through which KDM5 functions to regulate MB development, we generated a library of fly strains each bearing a conserved ID patient-derived *kdm5* missense mutation. We demonstrate that a subset of these strains presents with MB structural defects and deficits to long-term memory, as assessed *via* appetitive olfactory conditioning assays. Interestingly, fly strains bearing patient mutations that disrupt KDM5's histone demethylase activity do not present with MB morphological defects, yet have impaired learning and memory. These data suggest that KDM5 may function through demethylase-independent mechanisms to regulate MB development, independent of its effect on cognitive function. This is significant, as the prevailing model linking KDM5 dysfunction to ID assumes that altered demethylase activity of KDM5 is largely responsible for such deficits. We are currently investigating how KDM5 may utilize specific protein domains in coordination with Pros to guide neuronal development.

153 Neuronal mechanisms of neurofibromin dependent metabolic regulation *Valentina Botero*[1], Bethany Stanhope[6], Elizabeth Brown[2], Eliza Grenci[1], Tamara Boto[3], Scarlet Park[1], Lanikea King[1], Keith Murphy[1], Kenneth Colodner[4], James Walker[5], Alex Keene[2], William Ja[1], Seth Tomchik[1] 1) Scripps Research Institute, Jupiter, FL ; 2) Texas A&M University, College Station, TX; 3) Trinity College Dublin, Dublin, Ireland; 4) Mount Holyoke College, South Hadley, MA; 5) Massachusetts General Hospital, and Harvard Medical School, Boston, MA; 6) Florida Atlantic University, Jupiter, FL

Neurofibromatosis type 1 (NF1) is a genetic disorder predisposing patients to a range of complications, including benign tumor formations in the nervous system, altered cellular function, short stature, bone abnormalities, and increased rates of cognitive and developmental disorders. NF1 is caused by mutations in the *NF1* gene, which encodes neurofibromin (Nf1), a large protein that functions as a negative regulator of Ras signaling and mediates pleiotropic organismal and cellular functions. 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 locomotion and grooming activity. Activation of this restricted neuronal circuit mimics the loss of Nf1 function by increasing metabolic rate when stimulated. These data indicate that Nf1 may regulate changes in neuronal metabolic control, suggest that cellular and systemic metabolic alterations may be a pathophysiological mechanism of NF1, and provide a platform for investigating the cellular role of neurofibromin in metabolic homeostasis.

154 Traip controls brain size via suppression of mitotic DNA bridges *Ryan O'Neill*, Nasser Rusan National Heart, Lung, and Blood Institute, NIH, Bethesda, MD

Microcephaly is a failure to achieve proper brain size and neuron number during development. Most microcephaly-linked genes function either at the mitotic spindle or in DNA damage repair (DDR). Spindle proteins are thought to control brain size via their functions in neural stem cell (NSC) mitosis, whereas DDR proteins are thought to suppress DNA damage and cell death. However, since few microcephaly genes are well-studied in neurogenesis, we set out to characterize microcephaly genes in *Drosophila* brains. Here, we investigated the microcephaly gene *Traip*, known to function in DDR, and found that Traip promotes proper brain size by suppressing DNA bridges in mitosis. We show that *traip*$^{-}$ flies have microcephaly-like brain defects, including fewer neurons and marked loss of NSCs via caspase-dependent cell death. *traip*$^{-}$ NSCs have normal levels of DNA damage in interphase, suggesting that Traip does not fit the canonical model of DDR functions in microcephaly. In contrast, *traip*$^{-}$ NSCs are frequently aneuploid, multinucleate, or have micronuclei, suggesting mitotic failure. Live fluorescence microscopy of *traip*$^{-}$ NSCs revealed frequent mitotic DNA bridges, providing a possible explanation for the observed nuclear defects via chromosome fragmentation and cytokinesis failure. We characterized fluorescence-tagged *Traip* transgenes: in interphase, Traip is nuclear, whereas in mitosis Traip localizes on spindles, furrow, and midbody. A Traip variant lacking the nuclear localization signal is absent from the nucleus, but localizes properly in mitosis and rescues *traip*$^{-}$ brain phenotypes, showing that a mitotic *Traip* function is sufficient to suppress microcephaly. Together, our work challenges current thinking about the relationships between DDR, mitosis and microcephaly by showing that, instead of repairing DNA damage during interphase, Traip primarily functions to preserve NSC genome integrity by resolving mitotic DNA bridges. Now, using *Traip* as a model microcephaly gene, we are using whole brain imaging and 3D analysis to screen for suppressors and uncover downstream pathways that mediate microcephaly phenotypes. To date, we have found roles for MAPK and Toll signaling, abscission kinase, and caspase-dependent cell death. We are now testing whether these pathways also mediate the phenotypes of other microcephaly genes, including both DDR and mitotic spindle genes, and targeting these pathways as potential therapeutic targets to minimize neuron loss in microcephaly.

155 Dietary restriction ameliorates TBI-induced phenotypes in *Drosophila melanogaster*. *Rebecca Delventhal*[1,2], Emily Wooder[2], Maylis Basturk[2], Mohima Sattar[2], Jonathan Lai[2], Danielle Bolton[2], Gayathri Muthukumar[2], Matthew Ulgherait[2], Mimi Shirasu-Hiza[2] 1) Lake Forest College; 2) Columbia University Irving Medical Center

Traumatic brain injury (TBI) shares molecular and cellular hallmarks with neurodegenerative diseases (NDs), and is a major risk factor for developing neurodegeneration later in life. While our understanding of genes and pathways that underlie neurotoxicity in specific NDs has advanced, we still lack a complete understanding of molecular and physiological changes that drive neurodegeneration as an individual ages following a TBI. In recent years, many different studies have introduced *Drosophila* as a model organism for studying closed-head TBI, though they have primarily focused on acute or short-term outcomes of the TBI. In our research, we delivered a TBI to flies early in adult life, and then measured molecular and physiological phenotypes at short-, mid-, and long-term timepoints following the injury. We aim to identify the timing of changes that may contribute to progressive neurodegeneration, following a TBI. We confirmed prior work demonstrating a TBI-induced decline in lifespan, and uncovered evidence of a progressive decline in locomotor function, robust acute and modest chronic neuroinflammation, and a late-onset increase in protein aggregation. We also found evidence of metabolic dysfunction, in the form of starvation sensitivity and decreased lipids, that persisted beyond the immediate injury response, but resolved long-term. An intervention of dietary restriction (DR), or a low-protein diet, partially ameliorated some TBI-induced phenotypes, such as lifespan and locomotor function, though it did not alter the starvation sensitivity of injured flies. In the future, identifying molecular pathways altered in the short- or mid-term following TBI could suggest causality and point towards potential therapeutic targets.

156 Cyclin-dependent kinase 8 regulates mitochondrial morphology and modulates a Parkinson's disease model in Drosophila *Zhe Liao*[1], Kin Lam Wong[2], Esther Verheyen[1] 1) Simon Fraser University, Canada; 2) Stanford University Stanford, USA

Cyclin-dependent kinase 8 is a serine/threonine kinase, which functions in regulating RNA polymerase II mediated

transcription. Cdk8 forms a complex with Cyclin C, Mediator 12 (Med 12), and Mediator 13 (Med 13) that interacts with the core Mediator complex in a reversible fashion. While the regulatory role of Cdk8 in transcription is well-studied, we identified novel functions of Cdk8 in regulating mitochondrial morphology in Drosophila. When *cdk8* is knocked down ubiquitously, we observe phenotypic effects including held-up and droopy wing postures, reduced life span, and defects in both flight and climbing abilities. Surprisingly, the observed phenotypic effects are characteristics of flies with either PTEN-induced putative kinase 1 (*pink1*) or *parkin* mutations, which are two well-known players associated with Parkinson's disease (PD). Pink1 and Parkin normally function in regulating the homeostasis of mitochondria in a process known as mitophagy. Impaired or dysfunctional mitochondria will be recognized by Pink1 and Parkin and targeted for degradation by autophagy. Since tissue specific knock down of Cdk8 in either muscles or neurons also resulted in impaired climbing ability, we hypothesized that Cdk8 functions in a common pathway with Pink1 and Parkin in regulating mitochondria. Ectopic expression of Cdk8 in muscles significantly suppressed defects caused by the loss of function *pink1* allele, $pink1^{B9}$, including rescue of the thorax indentation phenotype which is due to muscle degeneration, and rescued climbing activity relative to $pink1^{B9}$ mutants. In addition, mitochondrial and muscle fiber morphologies were restored when Cdk8 was overexpressed in the $pink1^{B9}$ mutant background. Finally, we examined the effect of Cdk8 on mitochondria under physiological conditions. Expression of Cdk8 is tightly associated with mitochondrial dynamics in a kinase-dependent fashion, as fission-like alterations were found after elevating Cdk8 expression and fusion-like morphology was induced after depleting *cdk8* or expressing a kinase dead form of the protein. Together, we demonstrate that Cdk8 plays a novel function in regulating the homeostasis of mitochondria, and is able to revert the defective phenotypes caused by $pink1^{B9}$ mutant allele. Our findings suggest that modulation of Cdk8 may serve as a potential therapeutic target for patients with PD.

157 Endurance exercise ameliorates phenotypes in *Drosophila* models of Spinocerebellar Ataxias *Alyson Sujkowski*[1], Kristin Richardson[1], Matthew Prifti[2], RJ Wessells[1], Sokol Todi[2, 3] 1) Wayne State University School of Medicine Department of Physiology, Detroit, MI; 2) Wayne State University School of Medicine Department of Pharmacology, Detroit, MI; 3) Wayne State University School of Medicine Department of Neurology, Detroit, MI

Endurance exercise is a potent intervention with widespread benefits proven to reduce disease incidence and impact. While endurance exercise supports neural plasticity, enhanced memory, and reduced neurodegeneration, less is known about the effect of chronic exercise on the progression of movement disorders such as ataxias. Here, we focused on three different types of ataxias, Spinocerebellar Ataxias Type (SCAs) 2, 3 and 6, belonging to the polyglutamine (polyQ) family of neurodegenerative disorders. In *Drosophila* models of these SCAs, flies progressively lose motor function and accumulate levels of toxic SCAproteins. Excitingly, we observe dramatic protection of speed and endurance in exercised SCA2 flies and modest protection in exercised SCA6 models, while no benefit is observed in SCA3. Importantly, causative protein levels are reduced in SCA2 flies after chronic exercise, but not in SCA3 models, linking protein levels to exercise-based benefits. Currently, we are focusing on the activation of exercise-mimicking genes in SCA-model flies to define the mechanisms by which exercise preserves function in polyQ ataxias. The exercise-inducible protein *dSestrin* suppresses longitudinal mobility defects and improves early mortality in SCA2 flies, even without exercise. Furthermore, overexpression of *dSestrin* mimics exercise-induced reductions in disease protein in SCA2 flies by increasing autophagy. These improvements critically depend on previously-established functions of *dSestrin* that reduce oxidative damage and modulate mTOR activity. Our study suggests differential responses of ataxias to exercise, highlighting the potential for more extensive application of exercise-based therapies in the prevention of polyQ neurodegeneration.

158 Loss of function variants in *TIAM1* are associated with developmental delay, intellectual disability and seizures *Shenzhao Lu*[1,2,3], Rebecca Hernan[4], Paul Marcogliese[1,2], Yan Huang[1,2], Tracy Gertler[5], Meltem Akcaboy[6], Shiyong Liu[7], Hyung-lok Chung[1,2], Xueyang Pan[1,2], Xiaoqin Sun[7], Melek Oguz[6], Ulkühan Oztoprak[6], Jeroen de Baaij[8], Jelena Ivanisevic[5], Erin McGinnis[5], Maria Guillen Sacoto[9], Wendy Chung[4,10], Hugo Bellen[1,2] 1) Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX 77030, USA; 2) Jan and Dan Duncan Neurological Research Institute, Texas Children›s Hospital, Houston, TX 77030, USA; 3) Howard Hughes Medical Institute, Baylor College of Medicine, Houston, TX 77030, USA ; 4) Department of Pediatrics, Columbia University, New York, NY 10032, USA; 5) Division of Neurology, Department of Pediatrics, Ann & Robert H. Lurie Children›s Hospital of Chicago, Chicago, IL 60611, USA; 6) Department of Pediatrics, Dr. Sami Ulus Maternity and Children's Health and Diseases Training and Research Hospital, Ankara, Turkey; 7) Department of Neurosurgery, Xinqiao Hospital, Army Medical University, Chongqing, 400037, PR China; 8) Department of Physiology, Radboud University Medical Center, Radboud Institute for Molecular Life Sciences, Nijmegen, 6500HB, the Netherlands; 9) GeneDx, Inc., Gaithersburg, MD 20877, USA; 10) Department of Medicine, Columbia University Medical Center, New York, NY 10032, USA

The actin cytoskeleton is essential to maintain basic cell functions. Its dysregulation in neurons often leads to neurological diseases. T-lymphoma invasion and metastasis 1 protein (TIAM1) regulates Rac1 signaling pathways that affect the control of neuronal morphogenesis and neurite outgrowth by modulating the actin cytoskeletal network. To date, *TIAM1* has not been associated with a Mendelian disorder. Here, we describe five individuals with biallelic *TIAM1* missense variants who have developmental delay, intellectual disability, speech delay and seizures. We

found that the *Drosophila* ortholog of *TIAM1*, *still life (sif*), is expressed in larval and adult central nervous system (CNS), and is mainly expressed in a subset of neurons but not in glia. Loss of *sif* causes a severe reduction in viability, and the surviving adults exhibit climbing defects, are prone to severe seizures, and have a short lifespan. Both *sif* and *TIAM1* are toxic when ubiquitously overexpressed in *Drosophila*. Hence, either loss or gain of function of the gene affects development and survival. We assessed the toxicity associated with three *TIAM1* variants carried by two of the probands and compared them to the *TIAM1 reference* cDNA function *in vivo*. The data suggest that they are all loss-of-function (LoF) variants: *p.L862F* is the most severe LoF variant, while *p.R23C* and *p.G328V* are less severe LoF variants. In summary, we provide evidence that *sif* is important for appropriate neural function and that *TIAM1* variants observed in the probands are disruptive, thus implicating loss of *TIAM1* in neurological phenotypes in humans.

159 Extremely rare variants in *EIF4A2* are associated with a neurodevelopmental disorder characterized by hypotonia, intellectual disability and epilepsy *Maimuna Paul*[1,2], Anna Duncan[3], Casie Genetti[3,4,5], Michele Pinelli[6,7], Nicola Brunetti-Pierri[6,7], Alexandra Garza-Flores[8], Russell Saneto[9], Giuseppe Zampino[10], Emanuele Agolini[11], Ulrike Blümlein[12], Rami Abou Jamra[13], Raphael Carapito[14], Bertrand Isidor[15], Seiamak Bahram[14], Alyssa Ritter[16], Kosuke Izumi[16], Ben Pode Shaked[17], Ortal Barel [17], Bruria Ben Zeev[17], Hongling Pan[2,18], Hsiao-Tuan Chao[1,2,18,19,20,21], Pankaj Agrawal[3,4,5] 1) Department of Pediatrics, Baylor College of Medicine, Houston, TX; 2) Jan and Dan Duncan Neurological Research Institute, Texas Children's Hospital, Houston, Tx; 3) Division of Newborn Medicine, Boston Children's Hospital and Harvard Medical School, Boston, MA; 4) Division of Genetics and Genomics, Boston Children's Hospital and Harvard Medical School, Boston, MA; 5) The Manton Center for Orphan Disease Research, Boston Children's Hospital and Harvard Medical School, Boston, MA; 6) Telethon Institute of Genetics and Medicine, Pozzuoli, Italy; 7) University of Naples «Federico II», Pozzuoli, Naples, Italy ; 8) Department of Clinical Genetics, Cook Children's Hospital, Fort Worth, TX; 9) Departments of Neurology and Pediatrics, Seattle Children›s Hospital, University of Washington, Seattle, Washington; 10) Center for Rare Diseases and Birth Defects, Department of Woman and Child Health and Public Health, Fondazione Policlinico Universitario A. Gemelli, Rome, Italy; 11) Translational Cytogenomics Research Unit, Bambino Gesù Children's Hospital, IRCCS, Rome, Italy; 12) Department of Pediatrics, Carl-Thiem-Klinikum Cottbus, Germany; 13) Institute of Human Genetics, University of Leipzig Medical Center, Leipzig, Germany; 14) Laboratoire ‹ImmunoRhumatologie Moléculaire, Plateforme GENOMAX, INSERM UMR_S 1109, Faculté de Médecine, Fédération Hospitalo-Universitaire OMICARE, Fédération de Médecine Translationnelle de Strasbourg (FMTS), ITI TRANSPLANTEX NG, Université de Strasbourg, Strasbourg, France; 15) Service de Génétique Médicale, Hôpital Hôtel-Dieu, Centre Hospitalier Universitaire de Nantes, Nantes, France; 16) Division of Human Genetics and Metabolism, Department of Pediatrics, The Children›s Hospital of Philadelphia, Philadelphia, PA; 17) Pediatric Neurology Department, The Edmond and Lilly Safra Pediatric Hospital, Sheba Medical Center, Tel Hashomer, Israel; 18) Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 19) Texas Children's Hospital, Houston, TX; 20) Department of Neuroscience, Baylor College of Medicine, Houston, TX; 21) McNair Medical Institute, The Robert and Janice McNair Foundation, Houston, TX.

Eukaryotic Initiation Factor-4A2 encodes EIF4A2, an ATP-dependent RNA helicase subunit of the eIF4F complex, which recognizes the 5'cap structure of mRNAs and is required for mRNA binding to the ribosome. The fruit fly homolog *eIF4A* mediates the negative regulation of Decapentaplegic (Dpp) signaling. In the fly, Dpp-signaling regulates embryo patterning, eye and wing morphogenesis, and stem cell identity determination. The vertebrate homolog of Dpp, TGF-β/BMP, is a key regulator of neuronal differentiation, development, and function and is associated with various neurological disorders. Prior fly studies revealed that both gain and loss of function (GOF and LOF) *eIF4A* alleles modulate the rough eye and wing serration phenotypes associated with Dpp GOF and LOF, respectively. Despite the role of EIF4A2 homologs in key developmental processes, human disease-causing variants have not previously been identified. Here, we report eleven individuals with *extremely rare* variants in *EIF4A2* who all present with global developmental delay or intellectual disabilities, significant hypotonia, and epilepsy in most cases. To determine the pathogenicity of the *EIF4A2* variants *in vivo*, we generated flies expressing human *EIF4A2* wild-type (WT) and variants with a C-terminal HA tag for four *de novo* variants. We used the GAL4-UAS system to selectively express human EIF4A2 in fly neurons, wing, or eye. First, we conducted climbing assays to determine the impact of neuronal expression of EIF4A2 p.L344F, p.G364E, and p.T243I and found that these variants resulted in motor defects. Second, we found that the wing-specific expression of EIF4A2 p.T216I caused wing serration, which is consistent with loss of Dpp signaling. Third, we found that the eye-specific overexpression of EIF4A2 p.L344F, p.G364E, and p.T243I exacerbates the rough eye phenotypes associated with Dpp GOF, suggesting these variants are loss of function in nature. Finally, GMR GAL4 mediated knockdown of fly eIF4a using two different RNAi lines results in pupal lethality that can be rescued using the expression of human EIF4A2 WT. However, the EIF4A2 p.T243I and p.T216I variants fail to rescue the pupal lethality.
Together, these findings reveal that these *de novo EIF4A2* variants are pathogenic and alter fruit fly development in a dominant manner through either GOF or LOF mechanisms. Our work establishes a role for EIF4A2 dysfunction in human neurodevelopmental disorders.

168 Cactin, a component of spliceosome C complex, is required for collective border cell polarization and migration in the *Drosophila* ovary *Guangxia Miao*, Li Guo, Denise Montell University of California-Santa Barbara

Border cell migration in the *Drosophila* ovary serves as an *in vivo* model for the identification of molecular mechanisms that drive collective cell migration. While much is known concerning the physical, mechanical, and adhesive factors that steer the border cells and the cytoskeletal determinants of their morphology, less is known about the mechanisms that govern their initial delamination from the follicular epithelium and the coordination of individual cell polarization, morphology, and behavior. Here we report the identification of a gene, cactin, that is essential for these features of border cell migration. Cactin is conserved in evolution from yeast to man, yet has been ascribed to different biochemical activities in various cells, tissues, and organisms. We show that it is cactin's conserved spliceosome function that is required in border cells, rather than its reported effects on the Dorsal inhibitor Cactus, or its effect on Rac-mediated actin dynamics. Whole transcriptome analysis shows widespread alterations in splicing, and surprisingly, transcription read-through. Viability and cell fate specification were normal in Cactin knockdown cells, which exhibited individual cell mobility. However, Cactin knockdown caused specific defects in generating a dominant lead cell protrusion and in cluster cohesion. As a result, the cells frequently failed to delaminate. Apical polarity complex proteins were more tightly concentrated in each individual cell, and lost from apical cell-cell junctions and protrusions. These results elucidate a requirement for the spliceosome complex in preventing transcription readthrough and show cell-type-specific defects caused by depletion of a general splicing factor.

169 The cytoskeletal mechanics that shape a stem cell niche *Bailey Warder*, Kara Nelson, Justin Sui, Lauren Anllo, Stephen DiNardo University of Pennsylvania

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 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, 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 precise levels of AMC are required for niche shape. Further, live-imaging shows that proper niche shape is crucial for function. Current work addresses mechanisms that robustly polarize F-actin and MyoII in the niche. Interestingly, AMC can be regulated by mechanical forces exerted on a cell. I have evidence that GSC divisions are required for niche morphogenesis, and I suspect that AMC 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.** T32 GM007229 (BW), F32 GM125123 (LA), R35 GM136270 (SD)

170 Endocytic regulation of Fat protocadherins in tissue growth and morphogenesis Artem Gridnev, *Jyoti Misra* University of Texas at Dallas

Proper coordination of growth and morphogenesis during development is critical to formation of appropriately proportioned organs. The evolutionarily conserved protocadherins, Dachsous (Ds) and Fat constitute a signaling pathway that coordinates growth and morphogenesis by regulating the Hippo pathway and 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, an aspect that has been overlooked so far. 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.

171 Inter-organ signaling regulates the onset of myoblast fusion *Zhi-Rong Ruan*[1], Adwait Amod Sathe[2], Chao Xing[2], Elizabeth Chen[1] 1) Department of Molecular Biology, UT Southwestern Medical Center, Dallas, TX; 2) Eugene McDermott

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Myoblast fusion, in which mononucleated myoblasts fuse to form multinucleated myotubes, is a critical step in skeletal muscle development and regeneration. In *Drosophila*, myoblast fusion is initiated by the interaction between muscle cell type-specific adhesion molecules. Here, we show that ecdysone signaling provides an extrinsic cue to regulate the onset of myoblast fusion by activating the expression of a cell adhesion molecule (CAM). Ecdysone is a steroid hormone best known for transcription regulation during pupal metamorphosis via binding to the ecdysone receptor (EcR). Surprisingly, we found that mutation in an ecdysteroidogenic enzyme, Spook (Spo), caused partial myoblast fusion defects in *Drosophila* embryos. Spo was expressed in amnioserosa, a major source for ecdysone production in early embryogenesis. The fusion defect in *spo* mutant was rescued by expressing *spo* in amnioserosa, but not in muscle cells, demonstrating a non-autonomous function of ecdysone at the tissue level. Since ecdysone is also provided maternally, we further disrupted ecdysone signaling by expressing a dominant negative form of EcR in muscle cells of *spo* mutant, which led to a severe fusion defect. Interestingly, the expression of Dumbfounded (Duf), a CAM required for the initiation of myoblast fusion, was significantly decreased in the *spo;EcR^{DN}* mutant. Conversely, overexpressing Duf in the *spo;EcR^{DN}* mutant significantly rescued the myoblast fusion defect, demonstrating that Duf is a major downstream target of ecdysone signaling in muscle cells. Indeed, we identified EcR-binding sites in the promoter of the *duf* gene and showed by in vitro luciferase assays that these sites mediated transcription activation by EcR. Furthermore, the EcR-binding sites are adjacent to the binding sites of a muscle-specific transcription factor, Twist. EcR and Twist synergistically activated *duf* transcription in vitro and in vivo. Taken together, these findings reveal an indispensable role for the extrinsic ecdysone signaling in regulating myoblast fusion and identify *duf* as one of the direct targets of ecdysone signaling in muscle cells. In addition, our studies demonstrate that the extrinsic ecdysone signaling coordinates with the intrinsic transcription factor Twist to synergistically activate the expression of *duf* to control the onset of myoblast fusion.

172 Distinct contributions of ECM proteins to basement membrane mechanical properties in *Drosophila* *Uwe Töpfer*[1], Karla Yanín Guerra Santillán[1,2,3], Elisabeth Fischer-Friedrich[2,3], Christian Dahmann[1,3] 1) Institute of Genetics, Technische Universität Dresden; 2) Biotechnology Center, Technische Universität Dresden; 3) Cluster of Excellence Physics of Life, Technische Universität Dresden

The basement membrane is a specialized extracellular matrix (ECM) crucial for the development of epithelial tissues and organs. In *Drosophila*, the mechanical properties of the basement membrane play an important role in the proper elongation of the developing egg chamber, however, the molecular mechanisms contributing to basement membrane mechanical properties are not fully understood. Here, we systematically analyzed the contributions of individual ECM components towards the molecular composition and mechanical properties of the basement membrane underlying the follicle epithelium of *Drosophila* egg chambers. We find that the Laminin and Collagen IV networks largely persist in the absence of the other component. Moreover, we show that Perlecan and Collagen IV, but not Laminin or Nidogen, have a major contribution towards egg chamber elongation. Similarly, Perlecan and Collagen, but not Laminin or Nidogen, contribute towards the resistance of egg chambers against osmotic stress. Finally, we show using atomic force microscopy that basement membrane stiffness mainly depends on Collagen IV. Our analysis reveals how single ECM components contribute to the mechanical properties of the basement membrane controlling tissue and organ shape.

173 Affinity-driven germline-soma interactions mediate Drosophila oogenesis *Vanessa Weichselberger*[1,2], Ramya Balaji[1,3], Patrick Dondl[5], Anne-Kathrin Classen[1,3,4] 1) Hilde-Mangold-Haus, University of Freiburg, Germany; 2) Spemann Graduate School of Biology and Medicine (SGBM), University of Freiburg, Germany; 3) CIBSS Centre for Integrative Biological Signalling Studies, University of Freiburg; 4) BIOSS Centre for Biological Signalling Studies, University of Freiburg; 5) Department for Applied Mathematics, University of Freiburg, Germany

Germline-soma interactions are central to reproduction. In most vertebrate and invertebrate species, differentiating gametes are enveloped by a somatic epithelium, where the apical surface interacts with the germline cells. While signaling pathways acting on the two lineages have been described, little is known about how morphogenesis is coordinated between them. In the developing *Drosophila* egg chamber, the somatic follicle epithelium envelops germline-derived nurse cells and the growing oocyte to produce fertile eggs. We show that egg chamber morphogenesis can be divided into three major phases, that all require coordinated germline-soma morphogenesis. The expression of the transcriptional regulator Eya in follicle cells controls coordination between the germline and somatic follicle cells by differentially modulating affinity of the apical epithelial surface for nurse cells and oocytes. In all three phases, Eya expression patterns and levels are crucial to guarantee the right segregation of follicle cells over nurse cell and oocyte surfaces. Importantly, Eya-mediated affinity furthermore controls the spatial organization of nurse cells and the oocyte within the germline. Consequently, Eya is a master regulator of the soma-germline interface and through that controls all stages of egg chamber development.
Our work uncovers a new morphogenetic principle that enables the coordination of soma and germline and emphasizes the plasticity of epithelial behaviors during interactions with their adjoining tissues.

174 Genetic modifiers of NGLY1 deficiency identified through a *Drosophila* genetic screen point to the role of

NGLY1 in ERAD *Travis Tu'ifua*, Clement Chow Department of Human Genetics, University of Utah, Salt Lake City, UT

N-glycanase 1 (NGLY1) deficiency is a rare disease caused by autosomal recessive loss of function mutations in the *NGLY1* gene and is the only known congenital disorder of deglycosylation. 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, NGLY1's physiological significance in ERAD is not understood. One way to understand disease pathogenesis is to investigate the effects of genetic variation and modifier genes on disease presentation. 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 crossed this *Drosophila* model of NGLY1 deficiency with the *Drosophila* Genetics Reference Panel (DGRP), a collection of ~200 inbred fly strains with fully sequenced genomes, and scored for proportion of *NGLY1* knockdown flies surviving to adulthood. The genetic variation of the DGRP led to a spectrum of lethality ranging from strains that had 100% lethality to 100% viability. A genome wide association study (GWAS) generated a list of 61 candidate modifier genes of NGLY1 deficiency. Nine of these candidate genes encode ER resident proteins, proteins with known ERAD functions, or are involved with protein homeostasis. *CG31690* and *CG4341* were two independent unlinked hits in the GWAS that are orthologs of a single human gene, *TMTC2*, which encodes an ER adaptor protein involved with calcium homeostasis. *Hrd3* and *CG8405,* orthologs of *SEL1L* and *TMEM259*, were both hits and are known components of ERAD complexes that retrotranslocate misfolded proteins from the ER to the cytoplasm for degradation. We are functionally characterizing several of these candidate modifier genes of NGLY1 deficiency using *Drosophila* and cell culture models. The study of modifier genes can provide new insights into the etiology of the disease and functions of NGLY1, laying the foundation for the development of personalized therapeutics.

175 Identifying the genetic links between insomnia and cardiovascular disease using *Drosophila* models of sleep and cardiac physiology *Farah Abou Daya*[1], Torrey Mandigo[2,4], Shubhroz Gill[2,4], James Walker[2,4], Richa Saxena[2,3,4], Girish Melkani[1] 1) Division of Molecular and Cellular Pathology, Department of Pathology, School of Medicine, University of Alabama at Birmingham, Birmingham, AL, USA; 2) Center for Genomic Medicine, Massachusetts General Hospital, Boston, MA, USA; 3) Department of Anesthesia, Critical Care and Pain Medicine, Massachusetts General Hospital and Harvard Medical School, Boston, MA, USA; 4) Broad Institute, Cambridge, MA, USA

Insomnia is a common disorder defined by constant difficulty in falling and/or staying asleep. Studies have shown that insomnia is associated with cardiovascular disease (CVD) where insomnia confers more than a 2-fold increased risk of incident CVD. However, the specific shared causal pathways remain poorly understood, making it difficult to identify new therapeutic targets for insomnia that ameliorate CVD risks. Recently, our collaborators found 57 genome-wide significant genetic loci for insomnia. To identify causal genes under these association peaks and understand the mechanisms linking insomnia with CVD, we use *Drosophila melanogaster* models, which are well-established model systems for sleep and cardiac studies. We hypothesized that suppressing *Drosophila* orthologs of causal human insomnia genes will lead to compromised sleep and cardiac function. To assess the role of genes associated with the identified loci on cardio-physiology, we obtained RNAi stocks of 72 orthologs of genes under the 57 human genetic association peaks. Knockdown (KD) of these genes 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 3 to 7-day-old flies was monitored using the *Drosophila* Activity Monitoring System. Interestingly, our initial results show that 3 of 4 genes with fly orthologs, *ATPSynC*, *Bruce* and *Larsen*, within one insomnia genetic locus, show sleep and cardiovascular phenotypes. Mainly, cardiac-specific KD of *Larsen* led to significant cardiac dilation and reduced cardiac performance, while KD of *Bruce* led to significantly reduced cardiac performance without significant dilations. Moreover, KD of *ATPSynC* led to a compromised beating pattern with an elongated diastolic interval and shortened life span. Additionally, neuronal-specific KD of all 3 genes led to sleep fragmentation with only *Larsen* resulting in a significant decrease in overall sleep which was primarily due to a decrease in nighttime sleep. Assessments of inter organ crosstalk for this locus and measurements of sleep and cardiac parameters of other genetic lines are still in progress. This work will reveal mechanisms linking insomnia and CVD which would help prevent and treat both diseases.

176 A multi-model system approach identifies genetic interactions underlying atrial fibrillation susceptibility *James Kezos*, Anais Kervadec, Alex Colas, Chris Larson, Karen Ocorr Sanford Burnham Prebys Medical Discovery Institute, La Jolla, CA

Atrial fibrillation (AF), the most common heart rhythm disorder, is reaching epidemic proportions in the aging population. In both human and model systems, it is not understood how aging, genetic predispositions, and external environmental factors synergize to promote arrhythmia, nor which gene regulatory networks initiate and maintain AF. Over 200 genetic variants have been associated with increased AF susceptibility, suggesting that the underlying cause is multifactorial, involving networks of interacting genes. Resolving complex interactions modulating cardiac function

in AF is difficult in mammalian systems, but approachable using *Drosophila*. We utilize a multi-platform approach encompassing the genetically tractable *Drosophila* cardiac-aging model and hIPSC-atrial-like cardiomyocyte (ACM) model. The fly model allows for high-throughput quantification of aging effects in conjunction with genetic insults, whereas the ACM model allows for high-throughput combinatorial gene knockdown (KD). High-speed imaging of ACMs and fly hearts permit quantification of cardiac parameters such as action potential duration and arrhythmicity in ACMs, as well as contraction intervals and arrhythmicity in flies. Preliminary screens of candidate AF genes in both platforms have identified a network of seven corroborating hits. Network analysis has linked these 7 genes to ion channels, such as atrial-specific K+ channel *Shaker* (*Sh*), transcription factors *Dorsocross* (*Doc*) and *Pannier* (*Pnr*), as well as structural components such as *Sarcolamban* (*SclA*). Single genetic insults rarely produce robust arrhythmicity in our models, but we do see arrhythmia when testing interactions between genes in our network. Aging and pharmacological stressors (isoproterenol in ACMs, octopamine in flies) also interact with genetic insults to cause arrythmia. For example, KD of *Doc* in a *Sh* heterozygote background produces robust arrhythmicity, even at younger ages, whereas knockdown of *SclA* in a *Sh* mutant background mitigates cardiac alterations. Our approach has been validated in an atrial-specific double knockout mouse model of *Doc* and *Sh*, where atrial P-waves were deleted and QRS complex was broadened, both signatures of AF. Further identification and characterization of components from this *Shaker*-centric AF network across multiple platforms will not only improve our genetic and molecular understanding of AF pathogenesis but also guide novel experimental and therapeutic strategies to treat AF.

177 Exploring the effects of diet-induced obesity on the invasiveness of Drosophila tumours *Cecilia Cabrera*[1,2], Susumu Hirabayashi[1,2] 1) Medical Research Council (MRC) London Institute of Medical Sciences, London; 2) Institute of Clinical Sciences (ICS), Faculty of Medicine, Imperial College London, London

Epidemiological studies have shown that obesity promotes the development and progression of various cancers, including colorectal cancers. However, while cancer is a genetically heterogeneous disease, little is known about the genetic characteristics of tumours that associate with obesity. Here, we combine a series of *Drosophila* models of colorectal cancer with a *Drosophila* model of diet-induced obesity to explore whether and how tumours of different genetic profiles exhibit different invasiveness in response to obesity.

Using the dissemination of tumour cells into the abdominal cavity as a quantitative readout to assess tumour invasiveness, we demonstrate that obesity modulates the invasiveness of different tumours to different degrees. Furthermore, we found that tumours of different genetic profiles respond to obesity with different directionality; some tumours exhibited increased invasiveness in response to obesity, while other tumours were resistant to the effects of obesity.

RNA-Sequencing and comparative analysis of different tumours revealed Reactive Oxygen Species (ROS) as an important mediator that modulates the invasiveness of tumours in response to obesity. We demonstrate that dietary and genetic manipulation of ROS levels has a functional effect on the invasiveness of tumours.

Our observations indicate that the genetic profiles of tumours have a significant impact on the tumour's response to obesity. This highlights the importance of stratification of tumours in understanding the relationship between obesity and cancer. Our work also demonstrates the usefulness of *Drosophila* as a model system to study the connection between obesity, tumour heterogeneity, and tumour invasiveness.

178 The Clot Thickens: Tumor-induced coagulopathy is a conserved driver of host mortality *Katy Ong*, Tsai-Ching Hsi, David Bilder University of California, Berkeley, Berkeley, CA

Malignant tumors trigger a complex network of inflammatory and wound-healing responses, prompting Dvorak's characterization of tumors as 'wounds that never heal'. Some of these responses lead to profound clotting defects displayed by cancer patients, such as Disseminated Intravascular Coagulopathy (DIC), which correlate with poor prognoses. However, evidence suggests that lethality from coagulopathy is not fully explained by hemostatic dysregulation alone, raising the question of alternative mechanisms. Here, we demonstrate that tumor-induced coagulopathy is a paraneoplastic syndrome displayed by *Drosophila* as well as mammals. Fly tumors overproduce multiple components of the coagulation cascade, including the conserved Factor XIIIa homolog, and tumor-bearing flies exhibit abnormalities in clotting and wound healing that mirror DIC pathology. Critically, depleting coagulation components within the tumor significantly improved host survival, suggesting that coagulopathy is an unappreciated driver of mortality, even in insects that have an open circulatory system. We will discuss the underlying mechanism and the resulting insight into conserved responses of animals to malignancy.

179 Modeling Paraneoplastic Diabetes in *Drosophila* *Jyoti Tripathi*, Pradip Sinha Department of Biological Sciences and Bioengineering, Indian Institute of Technology Kanpur

Cancer patients display multiple metabolic disorders induced by humoral factors secreted by the growing cancerous

mass. Collectively termed as paraneoplastic syndromes, these systemic metabolic disorders often contribute to morbidity and even mortality of the patients: anorexia and cachexia being some of the cited examples. The onset of such paraneoplastic syndromes is marked by poor prognosis and often represents end-stage cancer. In certain cancers with the highest mortality rate, new-onset diabetes has been reported as another paraneoplastic phenomenon. Thus, management of cancer-induced diabetes remains a potential route to alleviate morbidity of cancer patients, improve their prognosis and, in certain instances, facilitate their early diagnosis and management. Here we show the systemic fallouts of oncogenic Ras signaling underlies cancer-linked diabetes in *Drosophila*. We found that a localized Ras activation in a non-vital organ decreases the adult eclosion rate. Further, oncogenic Ras signaling systemically perturbs host metabolism, particularly insulin signaling, with the accompanying onset of obesity, dyslipidemia, hyperglycemia, and, finally, insulin resistance and diabetes. Our results further reveal putative candidate secreted factors from the oncogenic Ras signaling cells as an inducer of these paraneoplastic syndromes. Our findings, therefore, reveal for the first time a causal underpinning of cancer-linked diabetes and the prospect of using *Drosophila* model for the exploration of therapeutic interventions.

180 Ionizing Radiation induces cells with past caspase activity that contribute to the adult organ in *Drosophila* and show reduced Loss of Heterozygosity *Sarah Colon Plaza*, Tin Tin Su 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. 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, increases the number of XPAC cells suggesting that *wingless* signaling regulates whether cells with caspase activity live or die. A dosage response curve shows that the number of XPAC reaches a peak and then decreases at higher radiation doses. 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.

181 Irradiation-Induced Cell Migration: An Epithelial-Mesenchymal Transition Process Regulated By Low-Level Caspase Activity *Lena Sapozhnikov*, Eli Arama The Weizmann Institute of Science

Apoptosis, the most common form of programmed cell death in animal development and homeostasis, is mediated by the activation of a unique family of proteases called caspases. However, an ever-growing list of studies suggest that caspases have an essential role in ensuring non-lethal cellular functions during normal development, tissue repair and regeneration, including in cell differentiation, proliferation, migration, signaling and cellular remodeling. Moreover, upon dysregulation, these caspase-dependent non-lethal cellular processes (CDPs) can also instigate disease. We previously discovered and characterized a novel non-apoptotic role of caspases in maintaining epithelial tissue integrity in *Drosophila*. We showed that upon ionizing irradiation or even spontaneously during development, epithelial cells compromised for caspase activity gain high migratory and invasive capacities. Furthermore, low levels of effector caspase activity, far below the threshold required to induce apoptosis, potently inhibit this process, which we termed irradiation-induced cell migration (ICM). Previous work showed that ICM is mediated by the DNA damage response (DDR) pathway, but the molecular mechanisms and signaling pathways underlying ICM downstream of the DDR remained largely elusive. Here we show that ICM shares many features with the process of epithelial-mesenchymal transition (EMT). Inactivation of key EMT-driving genes, which are also potential caspase substrates, including *twist* and *zfh1 (ZEB1)*, attenuates ICM. We further show evidence that the Notch signaling pathway is involved in ICM, and that effector caspases are important regulators of Notch-mediated ICM. Furthermore, members of the ADAM family of metalloproteinases, which among other functions are also responsible for the activation of the Notch signaling pathway, are also involved in ICM and may function as potential extracellular matrix modifiers during this process. Collectively, our findings indicate that ICM is an EMT-like process activated by genotoxic stress and is potently attenuated by non-lethal levels of caspase activity. Given that ionizing irradiation has been a common treatment for many cancers, and that one of the hallmarks of cancer cells is their ability to evade cell death, these findings may have implications for cancer malignancy and the induction/aggravation of metastasis in some patients undergoing radiotherapy.

182 A genome-wide CRISPR screen identifies DPM1 as a modifier of DPAGT1 deficiency and ER stress *Hans Dalton*[1], Raghuvir Viswanatha[2], Roderick Brathwaite, Jr.[2], JaeSophia Zuno[1], Stephanie Mohr[2], Norbert Perrimon[2,3], Clement Chow[1] 1) Department of Human Genetics, University of Utah School of Medicine, Salt Lake City, UT, USA; 2) Department

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Partial loss-of-function mutations in glycosylation pathways underlie a set of rare diseases called Congenital Disorders of Glycosylation (CDGs). Glycosylation is a broad category of sugar modifications on proteins and lipids, with functions ranging from complex post-translational modifications (e.g. N-glycosylation) to single additions involved in cell signaling (e.g. O-GlcNAc-ylation). CDGs have a range of symptoms, but commonly include severe epilepsy, developmental delay, and disability. CDG Type Ij is caused by loss-of-function mutations in *DPAGT1* – the first step in N-glycosylation. Our goal is to better understand the pathways connected to *DPAGT1* loss to develop potential treatment options.

We performed a CRISPR knockout screen using the drug tunicamycin (Tun), a potent inhibitor of DPAGT1 function, on *Drosophila* S2R+ cells. Loss of *DPAGT1* impairs N-glycosylation and causes massive protein misfolding, leading to reduction of cell surface glycoproteins and endoplasmic reticulum (ER) stress. We introduced a whole genome guide RNA library into S2R+ cells expressing constitutive Cas9. Cells were grown under either vehicle or Tun selection. Final populations were sequenced to determine candidate genes causing resistance or sensitivity to DPAGT1 inhibition. Gene Ontology of these genes related to the hexosamine pathway and glycolysis, two pathways important for N-glycosylation. We also performed a Concanavalin A (ConA) screen to assay cell surface glycoproteins in an identical pool of cells. This assay found loss of genes in GPI anchor biosynthesis (e.g. *PIG-A* and *PIG-H*) could rescue cell surface glycoproteins under DPAGT1 inhibition.

We created an *in vivo Drosophila DPAGT1* model where *Alg7*/*DPAGT1* RNAi is driven in the eye to cause a degraded eye phenotype. Using this and another ER stress model, we validated candidate genes via RNAi knockdown. RNAi in these models revealed that loss of the mannosyltransferase *Dpm1*, involved in mannose addition in all downstream glycosylation pathways, could strongly rescue *DPAGT1* inhibition and ER stress phenotypes. Testing its downstream pathways (O- and C-mannosylation [*rt*, *CG6659*]), N-glycosylation [*Alg3*], GPI biosynthesis [*PIG-M*]), we found loss of O-mannosylation, N-glycosylation, and GPI biosynthesis partially recapitulates the *Dpm1* RNAi rescue of *DPAGT1* inhibition and ER stress. These findings suggest impairment of *Dpm1*, or its downstream pathways, as key targets for potential therapeutics for DPAGT1 loss.

183 The Protein Phosphatase-1 regulatory subunit dPPP1R15 controls collective cell migration via the eIF2-alpha-ATF4-dependent ER stress pathway *Yujun Chen*, Jocelyn McDonald Kansas State University

Collective cell migration is essential for many developmental and pathological processes. *Drosophila* border cells migrate as a cohesive cluster during oogenesis and provide an excellent genetic model to elucidate conserved mechanisms of collective cell movement. Previously, we discovered that Protein Phosphatase 1 (Pp1) catalytic subunits are critical molecular regulators of border cell collective versus single cell behaviors. We now show that the conserved Pp1 regulatory subunit dPPP1R15 (also known as GADD34) plays a crucial role in border cell migration. Overexpression of dPPP1R15 causes border cells to round up and completely dissociate from the cluster during migration. These defects are rescued by overexpression of Pp1 catalytic subunits (Pp1c). Moreover, overexpressing PPP1R15 mutants that cannot bind to Pp1c no longer show migration or cluster-dissociation phenotypes. These data together indicate that dPPP1R15 functions through Pp1c in border cells. In contrast, RNAi-mediated knockdown of d*PPP1R15* prevents border cell delamination from the follicular epithelium. Live imaging reveals that, compared to control border cells, *dPPP1R15-RNAi border cells also extend* much smaller protrusions that are misdirected, thus contributing to the failure of border cells to migrate. The Pp1c/PPP1R15 complex is best known as a phosphatase complex that dephosphorylates eIF2alpha in the endoplasmic reticulum (ER) stress pathway. Indeed, loss of *dPPP1R15* elevates phosphorylation and activation of eIF2alpha and increases expression of the known downstream transcription factor, ATF4, in border cells. In addition, upregulation of PERK, a major eIF2alpha kinase, blocks border cell delamination and migration. Interestingly, depleting *PERK* by RNAi also delays border cell migration. Overexpression of a non-phosphorylatable eIF2alpha mutant, or knockdown of ATF4, significantly rescues *PPP1R15-RNAi* induced migration defects. Overexpression of a phosphomimetic eIF2alpha mutant, or of ATF4, impairs migration. Together, our data support the idea that restraining the PERK-eIF2alpha-ATF4 ER stress pathway via the dPPP1R15 phosphatase is critical for proper border cell migration. Our studies identify a new role for dPPP1R15 as a key regulator of collective cell behaviors through modulation of the ER stress response.

184 Increased intracellular pH promotes cell death in the developing *Drosophila* eye *Joanne Mendez*, Juan Pacheco, Daniel Orozco, Bree Grillo-Hill San Jose State University

Constitutively increased intracellular pH (pHi) is common to most cancers regardless of their tissue of origin or genetic background. Cancer research has traditionally focused on cancer-associated mutations and dysregulated signaling pathways. However much less is known about how changes in cellular chemistry, including pHi, regulate cancer cell behaviors. Our lab developed tools to increase pHi in the absence of other transforming mutations by over-expressing the *Drosophila* sodium-proton exchanger, *DNhe2,* in the *Drosophila* eye (*GMR>DNhe2*). We have found that *GMR>DNhe2* flies have a rough eye phenotype in adult flies. To determine the underlying cause of this rough eye,

we performed phenotypic analysis in larval and pupal eyes. We found the *GMR>DNhe2* flies have increased proliferation in larval eyes discs. Paradoxically, we found fewer interommatidial cells at the end of patterning in pupal eyes (11.4 compared to 15 cells per counting area in control). One aim of our current work is to perform a time course analysis of cell number in *GMR>DNhe2* flies to determine when in development these cells die, and to determine the mode of cell death. We next tested for genetic interactions between *DNhe2* and cell death genes. We found that the pH- dependent cell death is p53-dependent but caspase independent, which is inconsistent with apoptosis, but suggests autophagy. Second, we want to identify the genes that mediate this increased cell death at higher pHi. A dominant modifier screen identified overexpression of the oncogene *Myc* as a strong suppressor of the *GMR>DNhe2* rough eye. We tested whether this suppression is due to *Myc* attenuating the hyperproliferation effects of over-expressing *DNhe2*, but we saw no effect. Currently, we are quantifying the effects of co-expressing *Myc* and *DNhe2* on cell number in pupal eyes. Together, our findings will elucidate mechanisms for pH-regulation of conserved, critical developmental processes and provide evidence for new paradigms in growth control.

185 Lamp1 mediates lipid transport, but is dispensable for autophagy in *Drosophila* *Norin Chaudhry*[1], Margaux Sica[2], Satya Surabhi[2], David Sanchez Hernandez[2], Ana Mesquita[2], Adem Selimovic[1], Ayesha Riaz[1], Laury Lescat[2], Hua Bai[1], Gustavo C. MacIntosh[1], Andreas Jenny[2] 1) Iowa State University, Ames, IA, USA; 2) Albert Einstein College of Medicine, New York, NY, USA

The endolysosomal system not only is an integral part of the cellular catabolic machinery that processes and recycles nutrients for synthesis of biomaterials, but also acts as signaling hub to sense and coordinate the energy state of cells with growth and differentiation. Lysosomal dysfunction adversely influences vesicular transport-dependent macromolecular degradation and thus causes serious problems for human health. In mammalian cells, loss of the lysosome associated membrane proteins LAMP1/2 strongly impacts autophagy and cholesterol trafficking. Here we show that the previously uncharacterized Drosophila Lamp1 is a bona fide ortholog of vertebrate LAMP1/2. Surprisingly and in contrast to Lamp1/2 double mutant mice, Drosophila Lamp1 is not required for viability or autophagy, suggesting that fly and vertebrate LAMP proteins acquired distinct functions, or that autophagy defects in Lamp1/2 mutants may have indirect causes. However, Lamp1 deficiency results in an expansion of the acidic compartment in flies. Furthermore, we find that Lamp1 mutant larvae have defects in lipid metabolism as they show elevated levels of sterols and diacylglycerols (DAGs). Since DAGs are the main lipid species used for transport through the hemolymph (blood) in insects, our results indicate broader functions of Lamp1 in lipid transport. Our findings make Drosophila an ideal model to study the role of LAMP proteins in lipid assimilation without the confounding effects of their storage and without interfering with autophagic processes.

192 Enabling recombination on the 4th chromosome: FRT101F and Bloom syndrome helicase *Stuart Newfeld*[1], Mary Jane O'Connor[2], Samuel Goldsmith[1], Petra Tauscher[3], Samantha Daly[1], Osamu Shimmi[3,4], Michael O'Connor[2] 1) School of Life Sciences, Arizona State University, Tempe AZ ; 2) Dept. Genetics, Cell Biology and Development, Univ. Minnesota, Minneapolis, MN; 3) Institute of Biotechnology, University of Helsinki, Helsinki, Finland; 4) Institute of Molecular and Cell Biology, University of Tartu, Tartu, Estonia

Genes on the long arm of the Drosophila 4th chromosome are difficult to study because the chromosome lacks mitotic and meiotic recombination. Without recombination it is not possible to employ clonal analysis - the creation of a single mutant cell at a specific time and place followed by the phenotypic examination of its descendants. Here we report new resources for the 4th chromosome. For mitotic recombination we generated a chromosome with an FRT very near the centromere in 101F and a derivative that carries FRT101F with a distal ubiquitously expressed GAL80 transgene. This pair of chromosomes enables both unmarked and MARCM clones. An example from the IPC neurons of the brain is shown. For meiotic recombination we demonstrate that a *Bloom syndrome helicase* and *recombination defective* double mutant genotype can create recombinant 4th chromosomes via female meiosis. An example creating a w+ and y+ chromosome is shown. An efficient method for X to 4th transposition is also described. These chromosomes and strategies should accelerate the genetic analysis of protein coding genes on the 4th chromosome, including the 44 genes with no demonstrated function. Studies of these well conserved genes will close gaps in our knowledge of development and physiology.

193 Temperature-Inducible precision guided Sterile Insect Technique, TI-pgSIT *Nikolay Kandul*, Junru Liu, Omar Akbari Division of Biological Sciences, Section of Cell and Developmental Biology, UC San Diego, La Jolla, California, 92092 USA

Releases of sterile males are the gold standard for many insect population control programs, and precise sex sorting to remove females before male releases is essential to the success of these operations. To advance traditional methods for scaling the generation of sterile, fit, and competitive males, we previously described a CRISPR-mediated precision-guided Sterile Insect Technique (pgSIT). PgSIT functions by exploiting the precision and accuracy of CRISPR to simultaneously disrupt genes essential for female viability and male fertility. It utilizes a simple breeding scheme requiring two strains: one expressing Cas9 and the other expressing multiple guide RNAs (gRNAs). A single mating between these strains

mechanistically results in synchronous Cas9/RNA-mediated dominant biallelic knockouts of target genes throughout development, resulting in the complete penetrance of desired phenotypes in all progeny. This two-locus pgSIT design was used to engineer pgSIT systems in *Drosophila melanogaster, Drosophila suzukii*, a global pest of economically important soft-skinned fruits, and *Aedes aegypti,* the principal vector for arboviruses including dengue/yellow fever, chikungunya, and Zika virus. While effective at generating F_1 sterile males, the two-locus pgSIT depends on a genetic cross between the two parental strains which requires maintenance and sexing of two strains in a factory. Therefore, to further advance pgSIT by removing this crossing step, we describe a one-locus Temperature-Inducible pgSIT (TI-pgSIT) technology and demonstrate its proof-of-concept in *D.melanogaster.* Importantly, we were able to develop a true-breeding strain for TI-pgSIT that eliminates the requirement for sex sorting, a feature that may help further automate production at scale.

194 Seamless genetic engineering via CRISPR-triggered SSA allows spatio-temporal control of gene labelling *Gustavo Aguilar*[1], Milena Bauer[1], Alessandra Vigano[1], Carlos Jiménez-Jiménez[2], Isabel Guerrero[2], Markus Affolter[1] 1) University of Basel, Switzerland; 2) Centro de Biología Molecular Severo Ochoa (CSIC-UAM), Spain

Precise genome engineering is essential for both basic and applied research, permitting the manipulation of genes and gene products in predictable ways. The irruption of CRISPR/Cas technology rocketed the speed and ease by which exogenous sequences are integrated into specific loci. To this day, a number of strategies permit gene manipulation. Nevertheless, knock-in generation in multicellular animals remains challenging, partially due to the complexity of insertion screening. Even when achieved, the analysis of protein localization can still be unfeasible in highly packed tissues, where spatial control of gene labelling would be ideal. Here, we propose a method based on Homologous Directed Repair (HDR) and Single Stranded Annealing (SSA) repair pathways. HDR mediates the integration of a switchable cassette. Upon a subsequent CRISPR-triggered repair event, resolved by SSA, the cassette is seamlessly removed. We named the technique SEED/harvest. By engineering the Hedgehog pathway components, we demonstrated fast and robust knock-in generation with both fluorescent proteins and short protein tags in tandem. The implementation of short homology arms, further simplified and cheapen the process. Seamless knock-in generation can be achieved in as fast as one and a half months.
The use of SEED cassettes is not restricted the germ line. SSA can also be triggered in somatic cells, permitting conditional gene labelling. This is the first time CRISPR is used to trigger endogenous tissue-specific gene labelling. Since the technology is solely based on CRISPR, it can be easily used in combination with other tissue-specific CRISPR tools, as membrane labelling and generation of knock-outs. We provide evidence of cell-type specific endogenous labeling as well as temporal control of gene labeling, mediated by conditional expression of guideRNAs.
While our tools permit control of protein labeling with fluorescent proteins, the in vivo visualization and manipulation of proteins with short tags in tandem still represents a challenge. To complement the SEED technology, we have developed a toolkit based on rational nanobody engineering and functionalization that permits the visualization and manipulation of proteins tagged with short tags.

195 SpyChIP identifies genome-wide and cell type-specific transcription factor occupancy *Siqian Feng*, Richard Mann Jerome L. Greene Science Center, Columbia University, New York, NY

A major drawback of chromatin immunoprecipitation (ChIP) based techniques is the lack of cell type-specificity. To overcome this limitation, we developed SpyChIP to identify sites of cell type-specific transcription factor (TF) occupancy in native physiological contexts without tissue dissociation or nuclei sorting. SpyChIP takes advantage of a specific covalent isopeptide bond that rapidly forms between the 15 amino acid SpyTag and its binding partner, the 17 kD protein SpyCatcher. In SpyChIP, the target TF is fused with SpyTag by genome engineering, and an epitope tagged SpyCatcher is expressed by the Gal4/UAS system in cell populations of interest, where it covalently links to SpyTag-TF. Cell type-specific ChIP results are obtained by performing ChIP against the epitope on SpyCatcher using chromatin prepared from whole tissue.

Using SpyChIP, we characterized the genome-wide binding profiles of the Hox protein Ultrabithorax (Ubx) in two non-overlapping domains of the *Drosophila* haltere imaginal disc. Our results revealed extensive region-specific Ubx-DNA binding events, thus highlighting the significance of cell type-specific ChIP results and the limitations of whole tissue ChIP approaches. Analysis of SpyChIP results for Ubx provided novel insights into the relationship between chromatin accessibility and Ubx-DNA binding, as well as different mechanisms Ubx employs to regulate different downstream *cis*-regulatory modules (CRMs). We envision that the covalent bond between SpyTag and SpyCatcher will have wide-spread *in vivo* applications, and our demonstration of SpyChIP sets the stage for carrying out many other cell type-specific characterizations and manipulations *in vivo* that were previously unachievable.

196 The continuum of Drosophila embryonic development at single cell resolution *Xingfan Huang*[2,3], Ronnie Blecher-Gonen[1], Diego Calderon[2], Riza Daza[2], Stefano Secchia[7], Beth Martin[2], Baekgyu Kim[7], Alessandro Dulja[7], Cole Trapnell[2], Eileen Furlong[7], Jay Shendure[2,4,5,6] 1) The Crown Genomics institute of the Nancy and Stephen Grand Israel National Center for Personalized Medicine, Weizmann Institute of Science, Israel; 2) Department of Genome Sciences,

University of Washington, Seattle, WA ; 3) Paul G. Allen School of Computer Science & Engineering, University of Washington, Seattle, WA ; 4) Brotman Baty Institute for Precision Medicine, University of Washington, Seattle, WA; 5) Howard Hughes Medical Institute, Seattle, WA; 6) Allen Discovery Center for Cell Lineage Tracing, Seattle, WA; 7) European Molecular Biology Laboratory (EMBL), Genome Biology Unit, Heidelberg, Germany

Single cell technologies are a powerful new means to study metazoan development, enabling comprehensive surveys of cellular diversity at profiled timepoints, and shedding light on the dynamics of regulatory element activity and gene expression changes during the *in vivo* emergence of each cell type. However, nearly all such atlases of embryogenesis remain limited by sampling density*, i.e.* the number of discrete time points at which individual embryos are harvested. Given the rapidity with which molecular and cellular programs unfold, this limits the resolution at which regulatory transitions can be characterized.

To construct a continuous representation of embryogenesis *in vivo*, we would ideally sample embryos continuously. Although not possible with most model organisms, it is potentially possible in *Drosophila melanogaster*, where collections of timed and yet somewhat asynchronous embryos are easy to obtain. *Drosophila* could therefore serve as a test case to develop a framework for the inference of continuous regulatory and cellular trajectories as embryogenesis progresses. Of course, as *Drosophila* is a preeminent model organism that has yielded many advances in the biological and biomedical sciences, obtaining a single cell atlas of *Drosophila* embryogenesis is also an important goal in itself.

In this study, we report a continuous, single cell atlas of chromatin accessibility and gene expression that spans *Drosophila* embryogenesis. We profiled chromatin accessibility in almost one million, and gene expression in half a million, nuclei from eleven tightly staged, overlapping windows of 0 through 20 hours of embryogenesis. Leveraging the asynchronicity of embryos within each collection window, we developed a statistical model to estimate the age of each nucleus more precisely, resulting in continuous views of molecular and cellular transitions throughout embryonic development. From these data, we identify cell types, infer their developmental relationships, and link cell type-specific changes in transcription factor expression to changes in the accessibility of their cognate motifs. Looking forward, this strategy may facilitate future investigations of *in vivo* gene regulation throughout *Drosophila* embryogenesis at arbitrarily high temporal resolution.

197 Optogenetic manipulation of endogenous proteins in *Drosophila* by light-inducible trapping *Yineng Xu*, Bei Wang, Chun han Weill Institute for Cell and Molecular Biology and Department of Molecular Biology and Genetics, Cornell University

During animal development, the same molecular event can result in drastically different consequences depending on when and where it occurs. Thus, the spatial and temporal properties of signaling inputs and gene activity largely determine the biological outcomes. Spatiotemporal regulation is particularly important for neuronal development and function: Spatially, neurons occupy broad domains and may incur distinct signaling events at different cellular compartments as a result of unique interactions with the surrounding microenvironment. Temporally, neurons develop through distinct stages and require series of molecular events that occur in particular sequences. However, widely used methods for studying gene function, such as mutations, RNAi, and CRISPR, lack the spatiotemporal resolution required for dissecting fine developmental mechanisms. In contrast, light-controllable modules hold great promise for precise and instant manipulation of molecular events in cells. Towards this goal, we developed OptoTrap, a light-dependent protein trapping system, which builds on two light controllable systems—Cry2olig and magnets. This system forms large protein aggregates under blue light and can trap proteins that are tagged by GFP or split GFP. We generated several variants of this system for diverse applications in neurons and epithelial cells and characterized their association and dissociation properties. We demonstrate that this system can effectively trap GFP-tagged endogenous proteins of diverse sizes, subcellular locations, and functions. Functionally, light-dependent protein trapping of the septate junction protein Nrg in epithelial cells caused gain-of-function (GOF) and cell shrinkage, suggesting Nrg activation through clustering. In contrast, optogenetic trapping of kinesin heave chain (Khc) in somatosensory neurons caused effective loss-of-function (LOF), resulting in disruption of microtubule-dependent transport and dendrite reduction. Similarly, trapping of Nmnat in neurons caused dendrite reduction, likely due to disruptions of Nmnat's chaperon function. OptoTrap allows us to fine tune protein manipulation for graded phenotypes by changing illumination conditions, and thus offers great flexibility for dissecting protein functions in neuronal development. This toolkit should be applicable to broader developmental stages and diverse cell types for studying development mechanisms.

198 Spying on the dynamics of neuropeptides by the GRAB sensors in *Drosophila* *xiju xia*[1,2,3], Yulong Li[1,2,3,4] 1) State Key Laboratory of Membrane Biology, Peking University School of Life Sciences, Beijing, China; 2) PKU-IDG/ McGovern Institute for Brain Research, Beijing, China; 3) Peking-Tsinghua Center for Life Sciences, Academy for Advanced Interdisciplinary Studies, Peking University, Beijing, China; 4) Chinese Institute for Brain Research, Beijing, China

Neuropeptides are essential signaling molecules transported and secreted by large dense-core vesicles (DCVs). Neuropeptides play a critical role in many physiological processes, including brain development, sleep, circadian rhythm,

and feeding behaviors, alterations in neuropeptide levels have been associated to many pathological states, such as anxiety, stress and addition related disorders. For a precise understanding of neuropeptides' functions, it is important to probe when, where and how they are released in brain. Here, we developed a series of genetically-encoded green GRAB (GPCR activation based) sensors for detecting neuropeptides in living flies, including sNPF, CCha1, DH31 and FMRFa. These peptide sensors exhibited nanomolar to micromolar EC_{50} affinity and exquisite selectivity for cognate peptide ligands. Preliminary results suggested that transgenic expressing sNPF sensors are capable of detecting *in vivo* peptide release events triggered by high K^+ and optogenetic stimulation. Furthermore, calyx and horizontal lobe of mushroom body neurons (Kenyon cells) shows distinct release probabilities. Thus, these GRAB sensors provide sensitive and specific molecular probes to unravel the *in vivo* dynamics and molecular mechanisms of neuropeptide release in *Drosophila*.

Key words: neuropeptides, GRAB sensors, sNPF

199 Cryopreservation method for *Drosophila melanogaster* embryos *Li Zhan*, Min-gang Li, Amanda Neisch, Thomas Hays, John Bischof University of Minnesota, Minneapolis, MN

The fruit fly (*Drosophila melanogaster*), a foundational genetic model organism in the past century, has driven important biomedical science breakthroughs including six Nobel Prizes. However, more than 160,000 unique genotypes worldwide must be manually maintained through frequent and costly transfer of breeding adults to fresh food. In comparison, cryopreservation provides enormous advantages including protection against genetic drift, decreasing stock maintenance costs, and reducing the risk of stock loss caused by contamination or accidental mixing of precious stocks. Here we report an easily implemented and robust cryopreservation protocol of *Drosophila melanogaster* embryos, demonstrating potential for wide adoption. We present innovations for embryo permeabilization, cryoprotectant agent (i.e., anti-freezer) loading, and rewarming. After embryo collection, our protocol takes ~35 min to freeze and store the embryos in liquid nitrogen, ~25 min to thaw the embryo and remove the intra-embryonic cryoprotectant agent. We showcase its implementation in 25 distinct strains including wildtype and mutants. Using this protocol, >50% embryos hatch and >25% of the resulting larvae develop into adults (i.e., survival normalized to untreated embryos) after cryopreservation for most strains. For strains with initially low survival after cryopreservation, we show that the survival can be substantially improved by outcrossing to mitigate the effect of genetic background. After successive cryopreservation of 5 generations and 12-month storage in liquid nitrogen, the flies retain normal sex ratio, fertility and original mutation. Importantly, we find that non-specialists are able to use this protocol to obtain consistent results after training.

201 Minute mutations, cell competition, and cellular surveillance Nick Baker Albert Einstein College of Medicine

I will be describing my lab's work on cell competition and its physiological functions in Drosophila. Cell competition is the elimination of cells within an organ that only occurs when other cells are available to replace them. The term was originally coined to describe the elimination of 'Minute' (ie Rp/+) cells from mosaic imaginal discs containing wild type cells, which are progressively lost even though entirely Rp/+ animals are viable. Cell competition was originally envisaged as a homeostatic process responding to variations in cellular growth rate, but is now thought to provide a selection against abnormal cells that may arise in developing tissues. We used genetic screens to isolate mutations preventing cell competition. Analyzing these mutations changed how we think about the Minute phenotype, which iis now understood mostly to reflect a transcriptional response mediated by Xrp1, the key transcription factor that is expressed in Minute mutants. Remarkably, mutating this single transcription factor prevents cell competition and even restores normal overall translation to Minute cells. Minutes reduce translation though Xrp1-dependent phosphorylation of eIF2a by PERK, a kinase that also responds to ER stress, and not by reduction in ribosome numbers. Cell competition mutations do not affect organ size or reproducibility, instead they prevent certain kinds of abnormal cells from being eliminated. For example, aneuploid cells can be eliminated by cell competition on the basis of their abnormal Rp gene dose. Xrp1 seems also to be a common target of other cellular defects that trigger cell competition, including defects in ubiquitylation and protein turnover. Curiously, Xrp1 is a very rapidly evolving gene, which suggests it is involved in some evolutionary arms race, eg with pathogens. Interestingly, Xrp1 was first identified as a transcriptional target of p53 in the DNA damage response. Consistent with this, cells with different p53 activity levels compete with one another in imaginal discs. Notably, in mammalian cells Rp mutations lead to p53 activation, and there are multiple examples where different p53 activity levels lead to competition between mammalian cells. This suggests that aspects of mammalian p53 function may have been acquired by a p53 target gene in *Drosophila*, and leads us to speculate about how cell competition might contribute to cancer surveillance and tumor suppression.

202 Innate immune signaling sculpts neuron-glia interactions across lifespan Heather Broihier Case Western Reserve University, Cleveland, OH

Abstract is not available at the time of print.

203 Coping with mechanical stress: tissue dynamics in homeostasis and repair Yanlan Mao University College London

During growth and development, tissue dynamics, such as tissue folding, cell intercalations and oriented cell divisions, are critical for shaping tissues and organs. However, less is known about how tissues regulate their dynamics during tissue homeostasis and repair, to maintain their shape after development and upon wounding. In this talk we will discuss how tissues respond to mechanical perturbations, such as stretching or wounding, by altering their actomyosin contractile structures, to change tissue dynamics and cell shape, and thus preserve tissue shape and patterning. We will present new data on how changes in the 3D shape of cells upon wounding promotes efficient wound repair. We combine genetics, biophysics and computational modelling to study these processes.

204 The evolution of morphological novelties at the cellular and gene regulatory levels *Mark Rebeiz*[1], Shodja Donya[1], Rice Gavin[1], Vincent Ben[1], Smith Sarah[1,2], McQueen Eden[1,3] 1) University of Pittsburgh; 2) present address, Yale University; 3) present address, University of Michigan

The evolutionary origins of complex anatomical structures such as the eye or wing remain a major puzzle in evolutionary developmental biology. The development of morphology is controlled by gene regulatory networks (GRNs) composed of transcription factors, signaling pathways, and the regulatory sequences (enhancers) they control to regulate expression of structural genes that ultimately confer physical properties upon a tissue. To understand how new morphologies evolve, we strive determine the structure of their GRNs, localize evolutionary changes within these GRNs, and unravel how the network governs cellular behavior. I will present our work on the GRNs and cellular processes underlying new morphological structures in the genitalia of *Drosophila melanogaster* and its close relatives.

205 What long-term quantitative Imaging of stem-cells in their natural environment can tell us about the way they are born, differentiate, and talk to eachother Guy Tanentzapf University of British Columbia, Canada

Abstract is not available at the time of print.

206 Becoming an oocyte: demise of the germ cell program and new beginnings *Prashanth Rangan*[1], Kahini Sarkar[2], Noor Kotb [2], Shane Breznak[2] 1) Icahn School of Medicine at Mount Sinai; 2) University at Albany, SUNY

The continuity of sexually reproducing organisms depends on germ cells. Differentiating germ cells undergo meiosis to give rise to oocytes in females, which upon fertilization launches the next generation. Once the oocyte fate is specified, oocytes synthesize mRNAs and proteins, called the maternal contribution, that regulate development of the early embryo. My lab utilizes the *Drosophila* female germline to ask the following questions: How is germ cell entry into meiosis controlled? And what mechanisms eliminate the mitotic germ cell-specific programs upon acquisition of oocyte fate? My lab has discovered that production of viable oocytes depends on a stepwise progression through previously unidentified programmatic steps. First, that coordination of female germ cell differentiation and meiotic entry is governed by the male-specific lethal (Msl) complex member, Msl3, which licenses the expression of genes required for these programs. Further, we found that once germ cells have differentiated and committed to meiosis-I, genes critical for promoting differentiation are transcriptionally silenced by a feedback loop between heterochromatin and the Nucleopore complex. In addition, a cohort of perduring RNAs that promote germ cell differentiation and meiosis-I are removed by the No-Go Decay RNA surveillance pathway members. Finally, loss of transcriptional silencing or failure to remove perduring RNAs that promote differentiation and meiosis-I disrupts acquisition of the maternal contribution and causes loss of the oocyte fate. I will share our recent findings on the mechanisms regulating translation of meiotic proteins and the trigger that initiates silencing of the germ cell program.

207 Pioneers, settlers, and life on the OregonR trail: Transcriptional regulation during development *Melissa Harrison*, Meghan Freund, Marissa Gaskill, Tyler Gibson, Sam Krabbenhoft, Elizabeth Larson, Audrey Marsh, Eliana Torres-Zelada, Katherine Vietor University of Wisconsin-Madison

Coordinated changes in gene expression allow a single fertilized oocyte to develop into a complex multicellular organism. These changes in expression are controlled by transcription factors that gain access to discrete elements in the genome, allowing them to activate gene expression. While nucleosomes act as a barrier to transcription-factor occupancy, pioneer transcription factors have specialized functions that enable them to access binding sites within nucleosomes, establish accessible chromatin and facilitate binding of additional factors. My lab is particularly focused on how these pioneer factors act at the top of gene regulatory networks to control cell fate, including early embryonic reprogramming and stem-cell maintenance. By studying conserved developmental transitions using the powerful toolbox available for studies in *Drosophila*, we have begun to uncover shared properties of these pioneer transcription factors and identify barriers to their reprogramming functions. Our investigation of the pioneer factors Zelda, GAGA factor (GAF) and Grainy head and their roles in diverse tissues provides fundamental insights into how cell-fate transitions are controlled over development and how, when mis-regulated, can lead to disease.

208 Temporally dynamic antagonism between transcription and chromatin compaction controls stochastic photoreceptor specification Robert Johnston JOHNS HOPKINS UNIVERSITY

Nervous systems are extremely complex, with a myriad of neuronal subtypes defined by distinct functions, morphologies, and connectivities. Neuronal fate specification is driven by lineage, signaling, and stochastic regulatory inputs. The mechanisms controlling stochastic fate specification, in which a cell randomly chooses between two or more fates, are poorly understood. Studying the *Drosophila* retina, we characterized how natural variation in transcriptional regulation alters stochastic photoreceptor specification and color perception. We determined a mechanism in which simple interactions between transcription and chromatin compaction underlie this stochastic cell fate choice. Our findings suggest fundamental mechanistic principles underlying stochastic fate specification across species. Our work is revealing how cells harness molecular noise to control cell fate decisions in a metazoan.

209A Identifying potential caspase substrates involved in spermatid terminal differentiation in *Drosophila* *Tslil Braun*, Shmuel Pietrokovski, Eli Arama Department of Molecular Genetics, Weizmann Institute of Science, Rehovot, Israel

Caspases are the executioners of apoptotic cell death, but these proteases also promote a variety of non-lethal cellular process. The requirement of caspase activity for spermatid terminal differentiation in *Drosophila*, a process also known as spermatid individualization, as well as how these cells avoid death in the presence of active caspases, has been well established by our group and others in the past two decades. However, the precise role of caspases during the process of spermatid individualization is still unknown. Moreover, although dozens of caspase-dependent non-lethal cellular processes (CDPs) have been characterized so far, identifying the precise functions of caspases during these cellular processes remained largely elusive and has been highly challenging. Perhaps the main reason for the slow progress has been the need to identify specific caspase protein substrates and the consequence of their cleavage; in particular that caspase activity is restricted during CDPs and thus might only partially cleave some substrates.
To start addressing this question in spermatids, we first compiled a list of about 2,000 potential caspases substrates, mainly identified in several large proteomics studies of mammalian cultured cells undergoing apoptosis. We identified the *Drosophila* orthologs of these proteins and focused on those that are known to mediate cytoskeletal dynamics, a main feature of spermatid individualization, and in which the caspase cleavage site has been conserved. We then downregulated these genes in the male germ cells both in a wild type background and in the background of downregulation of the main effector caspase Drice. We then subjected the male flies to sterility tests. Of those, we mainly focused on the genes that produced synthetic sterility when downregulated together with *drice*, and which exhibited grossly normal spermatid maturation but failed to individualize. This approach yielded several potential caspase substrate candidates, which are now being further studied in depth for their roles during spermatid individualization.

210B Non-apoptotic activation of Drosophila Caspase-2/9 limits the growth of open-wound-like tumours by modulating JNK signalling and the tumour microenvironment *Luis Alberto Lopez*[1], Derek Xu [1], Kenneth Yamada[2] 1) University of Oxford; 2) National Institute of Dental and Craniofacial Research, NIH.

Personalised cancer therapy requires a deep molecular understanding of tumour features and the interplay between transformed cells and the immunological microenvironment. We have capitalized on a clinically relevant *Drosophila* tumour model, which simultaneously upregulates the EGFR and JAK/STAT signalling pathways (EJS tumours), to investigate the role of caspases in regulating tumour properties. Our results indicate that widespread non-apoptotic activation of initiator caspases limits JNK signalling and ensures cell fate commitment in EJS tumours. Additionally, caspase activation lowers the remote recruitment and *in situ* proliferation of EJS tumour-associated macrophages that fuel tumour growth. These findings uncover unrecognised tumour-suppressor activities of caspases that prevent exacerbation of cellular malignancy and tumour overgrowth without inducing apoptosis. Furthermore, they encourage the exploration of caspase-based therapeutic approaches against EGFR/JAK-STAT-activating tumours.

211C Differential sensitivity to cell death cues in long-lived, non-regenerative cells in the *Drosophila* hindgut *Jessica Sawyer*, Ruth Montague, Dongwon Lee, Don Fox Duke University

Quiescent long-lived cells often resist many types of cellular insults, which is an advantage in tissues with limited regenerative capacity. However, the molecular underpinnings that promote 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 cell death in a long-lived, low cell turnover organ. The *Drosophila* hindgut is comprised of three distinct segments: the diploid pylorus, the polyploid ileum, and the polyploid rectum. We have previously shown that ectopic expression of the pro-apoptotic genes *hid* and *rpr* causes cell death and regenerative hypertrophy in the pylorus. Here, we show that expression of *hid* and/or *rpr* does not activate a caspase sensor nor lead to cell death in the adult hindgut ileum or rectum. Polyploidy does not protect against cell death cues in these cell types, as ectopically expressing the caspase *dronc* (Caspase-9), often a downstream *hid/rpr* target, results in polyploid cell death. Surprisingly, the pylorus mostly resists cell death following *dronc* overexpression, which suggests that *dronc* expression alone is insufficient to kill pyloric cells. However, cell death in the pylorus depends on the canonical *hid/rpr/dronc* cell death pathway, as reducing the amount of *dronc* or increasing the levels of the *dronc* inhibitor *diap1* reduces *hid*-mediated cell death and regeneration in the pylorus. Collectively, these data suggest that canonical death signaling is active in the regenerative

pylorus, but distinct sensitivities to specific nodes of apoptotic regulation are present in each hindgut segment. We then compared our results to apoptotic gene expression in two other regenerative tissues: the larval eye and the adult midgut (both polyploid enterocytes and diploid enteroblasts). Both of these tissues behave like the regenerative pylorus. Specifically, *hid/rpr* overexpression robustly kills eye and midgut cells, while *dronc* overexpression does not. These data further underscore the distinct cell death signaling responses in the long-lived hindgut ileum and rectum. Finally, we find that the long-lived, *hid/rpr*-insensitive ileum and rectum lack a robust regenerative response to *dronc* killing, which may explain their distinct apoptotic responses. Our results argue that the *Drosophila* hindgut is an excellent model to reveal unique apoptotic regulation of long-lived epithelial tissues.

212A Knockdown of CG6191 (Mary Shelley) results in compensatroy apoptosis in the imaginal wing disc mediated through JNK signaling Jacob Kagey[1], *Razan El Yaman*[1,2], Ali Zamat[1,2] 1) Biology Department, University of Detroit Mercy; 2) ReBUILDetroit, University of Detroit Mercy

Drosophila share developmental pathways with humans and other organisms, and many of these pathways are disrupted in human tumorigenesis. We are studying the developmental expression patterns and knock-down phenotypes of the *Drosophila* gene *CG6191*, which we have named, *Mary Shelley* (*MS*). The human homolog of MS is Cables-1 and has been shown to be frequently downregulated in a number of human cancers, such as ovarian and endometrial. Using the MiMIC system, we find that MS expresses in a distinct band along the dorsal/ventral boundary compartment of the wing disc during larval development. The expression of MS is similar to the well-established expression pattern of the Notch and Wingless cell signaling pathway in the wing. To understand the developmental role that MS is playing in wing development, we utilized RNAi knockdown in the posterior compartment. We find that despite the RNAi confined to the posterior compartment, both the posterior and anterior compartments have a reduction in tissue size. This is due to increased apoptosis at the larval stage both autonomously (posterior) and non-autonomously (anterior). This pattern of non-autonomous apoptosis has previously been shown to be facilitated by the JNK signaling pathway. In MS knockdown cells we see an autonomous increase in the expression of the JNK ligand Eiger and a non-autonomous increase in Puckered, suggesting that the non-autonomous apoptosis is driven by aberrant JNK signaling. Currently we are utilizing a dominant-negative Basket allele to determine if the non-autonomous apoptosis observed is dependent upon JNK signaling. Understanding the downstream consequences of MS knockdown will help to better elucidate the developmental role of MS in Drosophila development.

213B Ribosome protein mutant cells rely on the GR64 cluster of gustatory receptors for survival and proteostasis in Drosophila Michael Baumgartner[1,3], *Alex Mastrogiannopoulos*[1], Iwo Kucinski[2,4], Eugenia Piddini[1] 1) School of Cellular and Molecular Medicine, University of Bristol, Biomedical Sciences Building, University Walk, Bristol BS8 1TD, UK.; 2) The Wellcome Trust/Cancer Research UK Gurdon Institute and Zoology Department, University of Cambridge, Tennis Court Road, Cambridge CB2 1QN, UK; 3) Perelman School of Medicine at the University of Pennsylvania, 3400 Civic Center Blvd, Philadelphia, PA, 19104; 4) Wellcome & MRC Cambridge Stem Cell Institute and Department 17 of Haematology, University of Cambridge, UK

Mutations in ribosome protein (Rp) genes and ribosome biogenesis factors result in debilitating diseases known as ribosomopathies. Recent studies in *Drosophila* have shown that cells heterozygous mutant for Rp genes (Rp/+) exhibit proteotoxic stress and aggregates, which drive stress pathway activation and apoptosis. Understanding how Rp/+ cells fend off proteotoxic stress could suggest mechanisms to ameliorate these and other conditions caused by proteotoxic stress. Here we find that Rp/+ epithelial cells express all six Gustatory Receptor 64 (Gr64) genes, a cluster of sugar receptors involved in taste sensation. We show that Rp/+ cells depend on Gr64 for survival and that loss of Gr64 autonomously exacerbates stress pathway activation and proteotoxic stress by negatively effecting autophagy and proteasome function in Rp/+ cells. This work identifies a non-canonical role in proteostasis maintenance for a family of gustatory receptors known for their function in neuronal sensation.

214C BMP-gated cell cycle progression drives anoikis during mesenchymal collective migration *Frank Macabenta*, Hsuan-Te Sun, Angelike Stathopoulos California Institute of Technology, Pasadena, CA

Organ integrity requires the elimination of abnormal cells to avoid compromised patterning and function. While cell competition has been characterized as a way to eliminate abnormal cells in epithelial tissues, little is known about how collectively-migrating mesenchymal cells maintain quality control. Here we investigate how lost cells within a migratory cohort are eliminated prior to organogenesis using the Drosophila embryonic caudal visceral mesoderm (CVM), migratory cells that ultimately form the midgut musculature. Through a combination of genetic manipulation, immunostaining, and confocal live imaging, we find that FGF signaling via *heartless* (*htl*) is critical to maintaining cell survival by antagonizing the cell death gene *head involution defective* (*hid*) specifically in the CVM, and that expression of *hid* is regulated in a cell-cycle dependent manner prior to myoblast fusion, ensuring that unfit cells are cleared before they can contribute to assembly. We additionally use a cell cycle-coupled degron system to show that precise coordination of the cell cycle is mediated by BMP signaling supported by CVM-expressed Tolkin (Tok) metalloprotease processing secreted Decapentaplegic (Dpp) ligand in the extracellular milieu. Mechanistically, we identified a BMP-

responsive enhancer that controls expression of the mitotic activator *string* (*stg*) in the visceral mesoderm. Through this study, we have demonstrated a system by which collectively migrating mesenchymal cells can control for migration errors via crosstalk between FGF and BMP signaling, and how errors in this system can result in significant consequences, including inappropriate invasion of other tissues.

215A Role of M1BP, a transcriptional pausing factor in JNK-mediated cell death during eye development *Hannah Darnell*[1], Anuradha Chimata[1], Madhuri Kango-Singh[1,2,3,4], Amit Singh[1,2,3,4,5] 1) University of Dayton; 2) Premedical Program, University of Dayton, Dayton, OH; 3) Center for Tissue Regeneration & Engineering (TREND), University of Dayton, Dayton, OH; 4) Integrative Science and Engineering (ISE), University of Dayton, Dayton, OH; 5) Center for Genomic Advocacy (TCGA), Indiana State University, Terre Haute, IN

In all multicellular organisms, transcriptional regulation is crucial to regulate differential gene expression, which is important during development and growth. Transcriptional pausing is one such mechanism used to control gene expression. Recently, we have shown that M1BP, a transcriptional pausing factor, promotes eye development by suppressing *wingless (wg)* expression. We showed that loss of M1BP induces ectopic caspase-mediated cell death that is triggered by *wg* induction. Blocking caspase-dependent cell death using p35 showed significant but incomplete rescue. M1BP is the functional homolog of ZKSCAN3, an autophagy repressor in humans. Jun-amino-terminal-(NH2)-Kinase (JNK) signaling is known to activate both caspase-dependent and -independent cell death. We hypothesized that M1BP could have a role in mediating multiple forms of cell death via JNK signaling during eye development. In our study, we have used the *Drosophila melanogaster* (Fruit fly) model to study the role of JNK pathway modulation during M1BP mediated eye suppression. Using the GAL4-UAS system, we modulated JNK signaling components along with downregulating *M1BP*. Here we present data that shows that the absence of *M1BP* results in activation of autophagic marker and JNK signaling. We also show that activation of JNK signaling enhances $M1BP^{RNAi}$ phenotype and downregulation of JNK signaling rescues the $M1BP^{RNAi}$ no eye phenotype.

216B PDZD8 promotes autophagy at ER-Lysosome contact sites to regulate synaptic growth *Rajan Thakur*[1], Kate O'Connor-Giles[1,2] 1) Department of Neuroscience, Brown University, Providence, RI; 2) Carney Institute for Brain Science, Providence, RI

Sites of apposition between organelles, referred to as membrane contact sites (MCSs), are hotspots for intracellular signaling, lipid metabolism, and organelle biogenesis/dynamics in eukaryotic cells. The endoplasmic reticulum (ER) forms an extensive and dynamic network of MCSs with almost all organelles. MCSs between the ER and endo-lysosomes are particularly abundant, suggesting important physiological roles. PDZD8 is an intrinsic ER transmembrane protein with a synaptotagmin-like mitochondrial lipid-binding protein (SMP) domain that has been reported to localize to ER-late endosome/lysosome and ER-mitochondria MCSs. PDZD8 is enriched in neurons. However, its role in the nervous system remains poorly understood. We identified Drosophila PDZD8 in a candidate screen for uncharacterized conserved regulators of synapse formation and function. We used the CRISPR-Cas9 system to generate null alleles and endogenously tag PDZD8. 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-lysosome MCSs. We show that activity-induced synaptic growth, neurotransmission, and locomotion are dysregulated in PDZD8 mutants, indicating important roles in nervous system development and function. We further show that PDZD8 regulates synaptic growth via autophagy. In PDZD8 mutants we see accumulation of autophagic proteins as well as autolysosomes. Our analyses suggest that PDZD8 is required for autolysosome maturation to promote autophagic flux in neurons.Overall, we propose that PDZD8-mediated ER-lysosome membrane interactions promote autophagy to regulate synaptic growth.

217C Investigating the contributions of Rab11 and the UPR in amyloid-β load at the *Drosophila* neuromuscular junction *Fatemeh Barmaleki Lighavn*, Faith Liebl Southern Illinois University-Edwardsville

Accumulation of abnormal aggregates formed by amyloid-β (Aβ) peptides and synaptic dysfunction are characteristics of Alzheimer's disease (AD). The accumulation of Aβ fragments during AD results in endoplasmic reticulum stress activating unfolded protein response (UPR) to restore proteostasis and can lead to synapse dysfunction and neurodegeneration. UPR is initiated by activation of stress sensors including the protein kinase R-like ER kinase (PERK), inositol-requiring enzyme 1 (IRE1), and activating transcription factor (ATF). UPR-inhibiting drugs are currently under investigation as a possible approach to modulate the metabolism of the Aβ precursor, amyloid precursor protein, and neuroplasticity. To assess the contribution of the UPR to Aβ load, we pharmacologically inhibited the UPR in a *Drosophila* AD model. Aβ levels were measured in AD model flies raised on STF-083010 and MKC-3946 (IRE1 inhibitors), GSK2656157 (a PERK kinase inhibitor), and the chemical chaperone Tauroursodeoxycholic acid. We find that AD model animals raised on 10 µM STF-083010 and 50 µM STF-083010 show decreased Aβ loads compared to control AD model animals. In another approach, we examined the effects of Rab11 on Aβ load. Rab11 is a GTPase that facilitates vesicle trafficking from the recycling endosome to the plasma membrane. Vesicle trafficking defects are characteristic of late-onset Alzheimer's disease. Our collective results will provide insight on the contribution of both Rab11 and the UPR to Aβ load and synaptic dysfunction.

218A The stress response transcription factor ATF4 regulates oocyte maturation *Lydia Grmai*[1], Emily Lackner[1], Hyung Don Ryoo[2], Deepika Vasudevan[1] 1) University of Pittsburgh; 2) New York University School of Medicine

Metazoans have evolved various stress response mechanisms to cope with cellular stress inflicted by external and physiological conditions. The Integrated Stress Response (ISR) is an evolutionarily conserved pathway that mediates adaptation to cellular stress via the transcription factor, ATF4. Here we describe a previously unknown role for *Drosophila* ATF4, encoded by the gene *cryptocephal* (*crc*), in oocyte maturation. We found that *crc* mutants have decreased egg laying and increased frequency of dorsal appendage defects in their eggs, with no apparent effect on fertilization rates. We observed substantial arrest in mid-oogenesis in *crc* mutant ovaries accompanied by premature apoptosis in early-stage egg chambers. Careful examination of ISR reporters revealed no detectable expression of ATF4 in the ovary, so we propose a non-autonomous role for ATF4 in the regulation of oogenesis.

Using a GFP insertion in the *crc* locus, we observed high levels of ATF4 expression in fat tissues surrounding the ovary. RNAi-mediated depletion of *crc* in fat tissues impaired oocyte maturation and decreased fertility similar to *crc* mutants. High levels of ATF4 expression in fat tissues suggest that this tissue has elevated basal levels of cellular stress. ATF4 activation in *Drosophila* can be mediated by one of two upstream ISR kinases: PERK, which responds to endoplasmic reticulum stress, or GCN2, which responds to amino acid deprivation. We are currently examining which of these kinases drives ATF4 expression in adult fat tissues to regulate oogenesis. Recent work has demonstrated that ATF4 acts as a coactivator for the Ecdysone receptor isoform EcR-B2 to regulate molting behavior. Since ecdysone signaling is a prominent regulator of oogenesis, we are currently parsing the role of ATF4-EcR-B2 signaling in fat tissues and its non-autonomous effects on oocyte maturation.

The *crc* 5' leader has multiple cis-regulatory elements called upstream open reading frames (uORFs) that typically influence translation of the main ATF4-encoding ORF. We previously demonstrated that *crc* translation is regulated by the translation reinitiation factors eIF2D and the DENR-MCTS1 heterodimer. Consistently, loss of these factors yields similar fertility defects, implicating translation reinitiation in fat tissues as a regulator of oogenesis. Together, our data indicate that stress signaling in fat tissues non-autonomously regulates oocyte maturation.

219B A *Drosophila* screen identified a role of histone methylation in ER stress preconditioning *Katie Owings*, Clement Chow University of Utah

Organisms face many stressors, and an ongoing challenge is understanding how an individual can respond to numerous insults over a lifetime. The accumulation of misfolded proteins results in cellular stresses, including endoplasmic reticulum (ER) stress. Many studies examine the ER stress response in isolation. In reality, cellular stresses rarely occur in isolation but often in the context of other stresses. Little is known about ER stress preconditioning, whereby conditioning with low levels of stress alters the ability to withstand subsequent ER stress. This project aims to use natural genetic variation to characterize ER stress preconditioning and its underlying mechanisms.

I began with an ER stress preconditioning screen that utilized the 200 *Drosophila* Genetic Reference Panel (DGRP) strains. Flies were subjected to heat shock (or no heat shock control), allowed to recover, placed on tunicamycin to induce ER stress until death, and survival was measured. Different genetic backgrounds led to a striking range in phenotypic responses to ER stress preconditioning, ranging from dying half as fast to 4.5 times faster with preconditioning than with no preconditioning. A genome-wide association study revealed that histone H3-K4 methylation is a solid potential mechanism of ER stress preconditioning. Several candidate modifiers have known roles in histone methylation. H3-K4 methylation marks promoters at transcribed genes and may play a role in transcriptional regulation. Two top modifiers, Pdp1 and TfIIA-L, are general transcription factors associated with RNA polymerase II. These hits solidify a potential role of transcriptional regulation underlying ER stress preconditioning. RNAseq was performed in the phenotypically extreme DGRP strains at different points in the preconditioning protocol to identify potential predictive gene expression signatures. Differentially expressed genes indicate a potential role of immune genes in ER stress preconditioning.

An effective ER stress response is critical for healthy development and aging. Disruptions in this response have been implicated in multiple human diseases, from diabetes to neurodegeneration. Understanding how previous stress events influence the ER stress response will provide insight into this pathway's fundamental biology and have important implications for therapeutic development.

220C Deciphering an unrecognized role of bZIP transcription factor IRBP18 during unfolded protein response (UPR) in *Drosophila* *Sahana Mitra*, Hyung Don Ryoo New York University

In eukaryotes, the endoplasmic reticulum (ER) serves as an essential organelle where membrane and secretory proteins undergo folding and maturation. Any physiological conditions that overwhelm the protein folding capacity of the ER could cause its dysfunction, widely referred to as "ER stress". The 'Unfolded protein response (UPR)" is a signaling mechanism that regulates gene expression in response to such ER stress. There are three classical UPR signaling

pathways mediated by ER stress sensors IRE1, PERK, and ATF6 respectively, which are conserved in *Drosophila*. The best-characterized effector of the PERK pathway is a bZIP transcription factor ATF4. The 5' UTR of ATF4 contains regulatory upstream open reading frames (uORFs) that block ATF4 expression in unstressed cells, but stimulates ATF4 translation specifically in response to PERK activation. We recently found that Xrp1 (bZIP transcription factor) is another effector of PERK that mediates an ATF4-independent branch of PERK signaling. Xrp1 mediates the induction of the anti-oxidant gene expression reporter, gstD-GFP, in response PERK activation. We found that Xrp1 also contains a regulatory 5' UTR with uORFs. Mutation of the last uORF causes a de-repression of the main ORF expression even in an unstressed cells. We further examined the role of *IRBP18,* a *Drosophila* homolog of *CEBPG* that is known to form a heterodimer with Xrp1 during the regulation cell competition and repair of DNA breaks. We find that the *IRBP18* is required for ER stress-induced gstD-GFP reporter expression. These results indicate that IRBP18 mediates an ATF4-independent branch of the unfolded protein response that may help maintain the redox equilibrium in cells under ER stress.

221A ER stress-induced JNK promotes stress granule formation via epigenetic modifications in *C9orf72* mediated ALS/FTD *Sahana TG*, Ke Zhang Mayo Clinic, Jacksonville, FL

Amyotrophic Lateral Sclerosis is a neurodegenerative disease affecting upper and lower motor neurons. A GGGGCC hexanucleotide repeat expansion (HRE) in the gene *C9orf72* is the most common genetic cause of ALS. Interestingly, *C9orf72* mutation also causes Frontotemporal Dementia (FTD), one of the most common early-onset dementia (collectively referred to as c9ALS/FTD). The HRE generates dipeptide protein repeats (DPRs) via noncanonical translation, resulting in five different DPR species. Among these species, the arginine-rich DPRs, poly(glycine-arginine, or GR), and poly(proline-arginine, or PR) are highly toxic and play a critical role in c9ALS/FTD pathogenesis. Stress granules (SGs) are RNA-protein condensates formed in eukaryotic cells upon stress. In c9ALS/FTD, these condensates recruit several RNA binding proteins namely TDP-43, FUS, hnRNP which can potentially form solid aggregates. However, how stress granules cause neurodegeneration is incompletely understood.
In an RNAi screen in a *Drosophila* model of c9ALS/FTD, we identified loss of *basket*, the fly homolog of c-Jun N-terminal kinase (JNK) to suppress neurodegeneration. JNK is a member of the mitogen-activated protein kinase pathway (MAPK). We found that the *basket*/JNK activity is upregulated in fly or cell models of c9ALS/FTD via the activation of ER-stress response protein IRE1/TRAF2. Furthermore, JNK hyperactivation transactivates G3BP1, a key SG assembly factor. Our results show that JNK promotes G3BP1 expression via epigenetic modulation of histone protein 3 (H3). Moreover, IRE1 or JNK inhibitors suppress H3 modification, G3BP1 protein levels, SG assembly, and survival defects in cells expressing poly(GR) or poly(PR). Hence, our data shows a unified connection between key ALS physiologies including ER stress, JNK/MAPK activation, and SG assembly which contributes to neurodegeneration.

222B Adenosine receptor and its downstream targets, Mod(mdg4) and Hsp70, work as a signaling pathway modulating cytotoxic damage in *Drosophila* *Michal Zurovec*[1,2], Yu-Hsien Lin[1,2], Houda Ouns Maaroufi[1,2], Lucie Kucerova[1], Lenka Rouhova[1,2] 1) Biology Centre, Inst Entomology, Ceske Budejovice, Czech Republic; 2) Faculty of Sciences, University of South Bohemia, Ceske Budejovice, Czech Republic

Extracellular adenosine (Ado) is an important signaling molecule involved in stress responses. Studies in various model organisms have shown that Ado is involved in "danger recognition" and tissue protection. However, the mechanism of Ado signaling is not well understood. In this study, we observed that *Drosophila* expressing the mutant huntingtin (Q93-mHTT) protein have reduced extracellular adenosine concentration in hemolymph. Using genetic methods, we altered Ado concentration, suppressed Ado receptor and Ado transporter function, and observed the effects of these changes on Q93-Htt fly viability. We found that overexpression of enzymes of Ado metabolism and suppression of Ado receptor (AdoR) or Ado transporter (ENT) can minimize mHTT-induced mortality. We also identified downstream targets of the AdoR pathway, the modifier of mdg4 (Mod(mdg4)) and heat shock protein 70 (Hsp70), which are able to modulate the formation of mHTT aggregates. Finally, we have shown that reduction of Ado signaling modulates some other responses to stress, including paraquat and heat shock treatments. Our study provides important insights into how Ado regulates stress responses in *Drosophila*

223C Investigating neuronal survival from caspase activity in neurodegeneration *Morgan Mutch*, Jiaqi Chen, Denise Montell University of California, Santa Barbara

The regulation of cell death and survival is critical for tissue development and function. Our lab has characterized a process named 'anastasis,' which is defined as cell survival following executioner caspase activation, previously thought to be the irreversible marker of apoptosis. Anastasis has been found to function during development, oncogenesis, and cell survival following injury in a variety of animal and cell culture models. Most anastasis research has focused on mammalian cancer cell lines and fly epithelial tissue, both highly proliferative cell types. However, the significance of anastasis has yet to be determined in the nervous system. Post-mitotic neurons have been shown to initiate apoptosis and activate executioner caspases without executing apoptosis in a neurodegenerative disease model. We propose that anastasis might enhance survival of neurons following stress like human Tau expression where it might function to slow neurodegeneration. I performed a loss of function screen in *D. melanogaster photoreceptor* neurons to test the functions

of genes that affect anastasis in proliferating wing imaginal discs for effects on neurodegeneration. The results suggest that the Insulin Receptor/Akt pathway, which drives cell proliferation, enhances Tau toxicity in the Tau-induced rough-eye model. I am currently investigating how Tau interacts with the Insulin Receptor/Akt pathway in fly photoreceptors, and how expression of signaling components changes Akt activity in a Tau background. In addition, I am exploring the role of Insulin Receptor signaling in regulating anastasis and apoptosis in a neurodegeneration model. I will present progress in understanding anastasis and genetic regulators of neurodegeneration in the fly nervous system.

224A Xrp1 and Irbp18 trigger a feed-forward loop of proteotoxic stress to induce the loser status Paul Langton[1], *Michael Baumgartner*[2], Remi Logeay[1], Eugenia Piddini[1] 1) School of Cellular and Molecular Medicine, University of Bristol, Bristol, UK; 2) Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA, USA

Cell competition induces the elimination of less-fit "loser" cells by fitter "winner" cells. In *Drosophila*, cells heterozygous mutant in ribosome genes, *Rp/+*, known as *Minutes*, are outcompeted by wild-type cells. *Rp/+* cells display proteotoxic stress and the oxidative stress response, which drive the loser status. Minute cell competition also requires the transcription factors Irbp18 and Xrp1, but how these contribute to the loser status is partially understood. Here we provide evidence that initial proteotoxic stress in *RpS3/+* cells is Xrp1-independent. However, Xrp1 is sufficient to induce proteotoxic stress in otherwise wild-type cells and is necessary for the high levels of proteotoxic stress found in *RpS3/+* cells. Surprisingly, Xrp1 is also induced downstream of proteotoxic stress, and is required for the competitive elimination of cells suffering from proteotoxic stress or overexpressing Nrf2. Our data suggests that a feed-forward loop between Xrp1, proteotoxic stress, and Nrf2 drives Minute cells to become losers.

225B Mechanisms for culling of *Drosophila* wing disc cells with loss-of-heterozygosity after irradiation *Jeremy Brown*[1], Inle Bush[1], Justine Bozon[1], Tin Tin Su[1,2] 1) Department of Molecular, Cellular and Developmental Biology, University of Colorado Boulder, Boulder, CO; 2) University of Colorado Cancer Center, Anschutz Medical Campus, Denver, CO

The ability of ionizing radiation (IR) to induce cell death is the basis for radiotherapy which is used to treat approximately half of cancer patients. IR causes DNA damage and events such as loss of heterozygosity (LOH). Undesirable effects such as oncogenesis can be promoted by functional loss of a tumor suppressor due to IR induced LOH. To study LOH events we use a cell-autonomous fluorescence-based system that uses the QF/QS transcriptional module. This allows our studies to expand into larval, pupal, and adult stages of *Drosophila* wing development. In parallel with QF/QS, we use the GAL4/UAS system to knockdown or overexpress genes of interest and we use engrailed-GAL4 to restrict UAS expression to the posterior compartment of the wing disc. In our studies, we compare the number and area of LOH clones found in the posterior to those of the anterior compartment after IR. Previously with these tools, we identified two distinct phases of LOH cell culling during development with p53-dependent as well as p53-independent mechanisms (Brown et al., PLoS Genetics, 2020, PMID: 33075096). Today, we will present recent data on LOH culling regarding genes involved with various cellular processes including Apoptosis-induced-Proliferation, ROS signaling, JNK and p53 signaling, and DNA repair.

226C An in vivo *Drosophila* screen to identify new regulators of ATF4 signaling Kristoffer Walsh NYU Vilcek Institute, New York, NY

In response to various physiological or external stresses, eukaryotic cells activate the Integrated Stress Response (ISR) to regulate protein synthesis and gene expression. ISR signaling begins when stress-activated kinases phosphorylate the translational initiation factor eIF2a, inhibiting general mRNA translation. Under these conditions, a small number of specially-regulated mRNAs – including that of transcription factor ATF4 – are translationally upregulated to activate a stress-induced gene expression program.

Our lab has previously demonstrated that cap-dependent translation inhibitor 4EBP is induced by ATF4 in the *Drosophila* fat body through the amino acid deprivation-activated ISR kinase GCN2. The 4EBP intron contains ATF4-binding sites that not only respond to environmental stress but also show physiological ATF4 activity during the development of larval fat body tissue. We developed a *Drosophila*-based ATF4 reporter that relies upon the 4EBP-intron ATF4-binding sites to drive the expression of dsRed, which we designated 4EBP-dsRed.

Using 4EBP-dsRed, I performed an in vivo RNAi screen to find new regulators of ATF4 in the *Drosophila* larval fat body. Among over 200 RNAi lines, 20 showed a decrease in the 4EBP-dsRed signal relative to wild-type larvae, implicating them as candidate positive regulators of ATF4. We have proceeded to validate these candidates through confocal microscopy and qPCR of endogenous 4EBP. By performing additional experiments in *Drosophila* tissue and cell culture, we aim to determine how these candidates regulate ATF4 and the ISR.

ISR dysregulation has been implicated in various diseases, including neurodegenerative disorders such as Charcot-Marie-Tooth disease. Identifying new positive regulators of ATF4 could provide a greater understanding of the ISR, potentially

aiding the development of new drugs and other therapeutics.

227A Characterizing the landscape of alternative splicing events regulating the clearance of nurse cells by non-professional phagocytes in Drosophila melanogaster oogenesis *Shruthi Bandyadka*, Diane PV Lebo, Ana Fiszbein, Kimberly McCall Boston University

The death and clearance of nurse cells marks an important developmental milestone in Drosophila melanogaster oogenesis. A subset of the Main Body Follicle Cells (MBFCs) that encapsulate the oocyte and the nurse cells within the egg chamber undergo marked cytoskeletal remodeling to become Stretch Follicle Cells (SFCs). Towards the final stages of oogenesis, the nurse cells dump their contents into the oocyte and are subsequently induced to die by the phagocytosis machinery of SFCs. This non-autonomous, phagocyte-dependent form of cell death is accomplished by the SFCs migrating into the spaces between the nurse cells and acidifying them externally by employing proton-pumping Vacuolar-ATPases. In this study, we characterize the co-transcriptional events that precipitate the transition of columnar, non-phagocytic MBFCs into squamous, phagocytic SFCs, including those that orchestrate the engulfment and processing of nurse cells. We focus on differential expression at the transcript level arising from alternative splicing, which significantly amplifies the transcriptomic repertoire, and thereby the proteomic diversity of tissues.
To capture the incidence and diversity of splicing events between MBFCs and SFCs at the genome-level, we sequenced the mRNAs undergoing translation by immunoprecipitating GFP-tagged ribosomes using Translating Ribosome Affinity Purification (TRAP-seq). We analyzed the short-read RNA-seq data using HITindex to identify terminal exons that could act as hybrid internal exons, and LeafCutter to identify intron excision events and novel splice sites.
We observed that hundreds of terminal exons in MBFCs were classified as hybrid internal exons in SFCs. Gene ontology analysis revealed that a plurality of genes with hybrid first exons had important physiological functions such as cytoskeletal, trans-membrane, or secreted proteins and were involved in major signaling pathways, including the immune-related Imd and TNF-alpha pathways. Specifically, we discovered that the first exons of Vacuolar-ATPases subunit Vha100-2 and kinase Src42A in MBFCs were used as internal exons in SFCs. Additionally, we found statistically significant exon skipping events in the calcium-binding protein Mlc1 and in Sec23, which is involved in vesicle trafficking in autophagy.
In summary, we have identified novel regulatory events orchestrating nurse cell clearance that are not captured at the gene-level, indicating that co-transcriptional regulation of gene expression could play a more prominent role in cell death and clearance than was previously appreciated.

228B Wdr59 regulates the interaction of GATOR1 with RagA to inhibit TORC1 activity in the *Drosophila* ovary *Yingbiao Zhang*, Chun-Yuan Ting, Shu Yang, Mary Lilly National Institute of Child Health and Human Development, NIH, Bethesda, MD

TORC1, a master regulator of metabolism, is dysregulated in a wide array of human diseases. The GATOR complex is an upstream regulator that controls the activation of TORC1 in response to nutrient stress. The GATOR complex is comprised of two subcomplexes, the GATOR1 complex (Iml1, Nprl2, Nprl3), inhibits TORC1 activity by serving as a GAP for the TORC1 activator RagA, a component of the lysosomally located Rag GTPase. Conversely, the GATOR2 complex opposes the activity of GATOR1, thus serving to promote TORC1 activity. Current models developed from studies in mammalian tissue culture cells suggest that the GATOR2 complex contains 5 components, Mio, Wdr24, Seh1, Sec13 and Wdr59, TORC1 activity is down regulated by loss function of any one of these components. In *Drosophila*, loss function mutants of *mio*, *seh1* and *wdr24* exhibit decreased TORC1 activity and cell growth in the female germline. Surprisingly, we find that deletion of the Wdr59 gene lead to the opposite phenotype. Specifically, *wdr59* mutants have increased TORC1 activity and ovarian growth and decreased levels of autophagy. Our data support a model in which Wdr59 harbors neither GATOR2 nor GATOR1 function, but act as a positive regulator of GATOR1 by inhibiting the binding affinity of RagA to the GATOR1 complex in *Drosophila* germ cells.

229V Toxicological study and genetic basis of BTEX susceptibility in *Drosophila melanogaster* *Temitope Adebambo*[1,2,3], Don Fox[2], Adebayo Otitoloju[3] 1) Emory University School of Medicine; 2) Duke University, Durham, NC; 3) University of Lagos, Nigeria

Drilling and marketing operations in the oil and gas sector has led to the environmental release of benzene, toluene, ethylbenzene and xylene (BTEX) causing harmful effects to man and other environmental components. Drosophila melanogaster has established protocols that can be used to study the effect of these chemicals and the genome wide mechanism underlying their toxic actions. In this study, the toxicological profile of benzene, toluene, ethylbenzene, p-xylene, m-xylene, and o-xylene in Drosophila melanogaster was evaluated in adult flies and first instar larvae of white w1118 strain. The impact of fixed concentrations of benzene and xylene on apoptosis and mitosis were also investigated in adult progenitor tissues (imaginal discs) found in the third instar larvae. In addition, Genome Wide Association Screening (GWAS) of the Drosophila Genetic Reference Panel (DGRP) was conducted to identify genes that are critical for toxicological responses in Drosophila melanogaster for p-xylene. The results of the toxicity tests showed that toluene and p-xylene are the most toxic chemicals to adult flies with LC50 ≥ 0.166 mM, while a significant and dose-dependent

decrease in fly eclosion was observed with benzene, p-xylene, and o-xylene. A significant increase in apoptotic markers and Phospho-Histone-3(PH3) activity was also observed in the imaginal wing discs of larvae exposed to benzene and p-xylene. The genome-wide analyses revealed few single nucleotide polymorphisms (SNPs) with very low p-values (p≤10-5) in 38 regions of Drosophila melanogaster genome as critical for responses to p-xylene. This study reveals the strength of Drosophila melanogaster genetics and apoptotic-proliferative ability of the imaginal disc as an accessible approach to study BTEX compounds.

230V Symptoms following Traumatic Brain Injury in a Drosophila melanogaster CTE Model are Ameliorated by Ketone Body Enantiomers *Katelyn Mooney*[1,2], Geoffrey Tanner[1,2], Jeremy Balsbaugh[1], Kate Gavilanes[1], Dariana Mota[1], Joseph Mooney[1], Mirsha Pierre[1], Alana Grant[1], Hanna Val Pelt[1], Yves Thelusma[1] 1) University of Connecticut; 2) The Connecticut Institute for Brain and Cognitive Sciences

Traumatic Brain Injury (TBI) events—which are increasing in both concern and incidence in contact sports—are defined as brain injuries sustained from a blow or force to the head that disrupts normal brain function. Following TBI, individuals may experience headaches, confusion and disorientation, and difficulty speaking. Multiple TBI events may lead to Chronic Traumatic Encephalopathy (CTE), a progressive neurodegenerative condition characterized by impaired judgment and learning and memory, and personality changes including anxiety, depression, and hyper-aggressive behavior. Progressively more young athletes in contact sports are experiencing repeated TBI, making research into TBI and CTE critical.
The ketogenic diet (KD) is a low-carbohydrate, adequate-protein, and high-fat diet that has been used effectively in the clinic to treat neurological disorders such as intractable pediatric epilepsy. We have shown that directly supplementing standard high-carbohydrate Drosophila melanogaster diets with ketone bodies (KBs; specifically, beta-hydroxybutyrate, BHB) increases lifespan and decreases male-on-male aggression following TBI events. Our results have suggested that different enantiomeric forms of exogenous BHB may vary in their effectiveness at reducing aggression and increasing lifespan. Compared with a control diet (CD) alone, dietary supplementation with either pure R-BHB (the main endogenous form), or a BHB racemic mixture, both significantly decrease number of post-TBI aggressive events in male-male pairs; racemic and pure-S-BHB lengthen latency to the first aggressive event.
The mechanism by which BHB supplementation ameliorates elevated aggression and increases lifespan following TBI is poorly understood. BHB's primary metabolic function is mitochondrial ATP production under conditions of low circulating glucose levels leading to elevated brain-wide ATP/ADP ratios. However, BHB can also act as a GPCR ligand, a gene-expression regulator (via histone deacetylase inhibition), and a sirtuin protein activator (via increased cytoplasmic [NAD+]). Sir2 activation promotes cell survival through a variety of mechanisms, including activation of autophagy proteins (e.g. autophagy-related protein 8a; Atg8a) which can mediate clearance of damaged cellular components. We hypothesize that BHB works to decrease neuronal cell death following TBI by improving neuronal metabolic stability and by activating and upregulating levels of Sir2 and Atg8a.
Future experiments will include confirming reduced neuronal cell death, studying the ability of BHB to rescue learning following TBI, and determining NAD+/NADH ratios in whole brains from TBI-subjected flies receiving exogenous BHB.

231V Roles of Hippo and Ecdysone Receptor Signaling in the regulation of *dronc* *Karishma Gangwani*[1], Amit Singh[1,2], Madhuri Kango-Singh[1,2] 1) Department of Biology, University of Dayton, Dayton OH; 2) Integrative Science and Engineering Center, University of Dayton

The Hippo pathway coordinately controls the essential growth regulatory processes of cell proliferation and death, and is required for regulating organ size in both flies and humans. Downregulation of Hippo signaling results in tissue overgrowth and tumorigenesis, whereas hyperactivation of Hippo signaling results in caspase-mediated cell death. Caspases are major regulators of apoptosis and suppression of caspase dependent apoptosis is often linked to aberrant growth. Further, JNK activation or hyperactivation of Hippo pathway by Mst1 (*Drosophila* Hippo) overexpression or YAP (*Drosophila* Yorkie, Yki) knockdown causes mitochondrial damage that induces Caspase-9 mediated apoptosis in cancer cell lines. Thus, caspases play a role in maintaining tissue homoeostasis and in diseases like cancer. Previously, we reported that the initiator caspase *Drosophila* Nedd-2 like caspase (*dronc*), Caspase-9 homolog, is a transcriptional target of the Hippo pathway in *Drosophila*. We showed that *dronc* transcription requires the growth inhibitory Hippo component Warts (Wts). Developmental apoptosis is controlled by small lipophilic hormones like ecdysone. Earlier studies have shown that *dronc* expression is regulated by the Ecdysone receptor (EcR) signaling pathway and an EcR regulatory element has been identified on the *dronc* promoter. Recently, Wts was shown to regulate Ecdysone production through Yki and the microRNA Bantam, suggesting a feedback mechanism involving Hippo and Ecdysone pathways. We found that depletion of Scalloped (Sd, TEAD in mammals) or Ecdysone receptor (EcR) or their corepressors resulted in derepression of *dronc* expression suggesting that *dronc* may be regulated by a default repression mechanism. Other preliminary data suggest that *dronc* regulation may involve cooperative interactions between Sd and EcR. Although an EcR binding site is mapped to the *dronc* promoter, the role of Sd has not been characterized. Using qRT-PCr based approaches we confirmed that dronc expression is regulated by Yki/Sd and EcR. Further, we identified a potential Sd site on the dronc promoter. Here, we present our work on the regulation of *dronc* by the Hippo and EcR signaling pathways,

and its implications on development.

232V JNK-independent Eiger/TNFR signaling during cell competition *Aditi Sharma Singh*, Laura A. Johnston Columbia University Medical Center, New York, NY

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. Such competitive interactions can benefit the tissue by eliminating unhealthy cells, but can also be detrimental when oncogenic cells take over an otherwise healthy tissue in a process known as super-competition. 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. In this model, 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 loser cells, consisting of Toll-related receptors, the secreted ligand Spaetzle, and NF-kappaB proteins. Our recent genetic evidence indicates that Eiger (Egr), the sole TNF in *Drosophila*, and its cognate receptor, Grindelwald (Grnd)/ TNFR, also contribute to the elimination of WT loser cells. Egr/Grnd signaling requires the TNFR adaptors Traf4/6 to kill the loser cells, but not Tak1 or other canonical downstream JNK effectors, indicating that in the competitive context, Egr functions independently of JNK activity. Moreover, although Grnd is expressed in all wing disc cells, *in-vivo* studies with Egr reporters and GFP fusion proteins reveal no evidence of Egr expression in either loser or winner cells. However, Egr, produced as both a secreted and transmembrane protein, is expressed in multiple tissues in growing larvae, so could remotely contribute to loser cell elimination. To determine which cells/tissues produce Egr during cell competition, we are selectively depleting *egr* from each expressing tissue while concomitantly inducing competitive interactions in wing discs. In addition, we are carrying out experiments to determine the mechanism by which Egr/Grnd activity intersects with the CCSM. Our experiments will provide insight into how local and remote signals converge to influence cell-cell interactions in the growing wing disc during Myc induced super-competition.

233V Utilizing Live Cell Imaging in *Drosophila melanogaster* Salivary Glands to Determine if Resveratrol Treatment Activates Heat Shock Factor DNA Binding *Martin Buckley*, Nichole Webb, Riley Bricker, Tyra Skalos, Stacy Hrizo Slippery Rock University of Pennsylvania

Oxidative stress is a hallmark of many aging diseases. There is great interest in understanding the mechanism of action of anti-oxidant treatments and their effects on the cell. One major stress response pathway is the heat shock response (HSR) that 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, reductants and other chemical stresses. HSF activates expression of the Hsp70 chaperone, which helps cells deal with protein folding stress. We examined the effect of feeding the anti-oxidant, resveratrol on the ability of wildtype Drosophila to withstand heat stress. Treatment with 100 and 400uM resveratrol increased the ability of the flies to withstand heat stress. One possible reason for this is the flies had increased HSF activity due to the resveratrol treatment. To examine this hypothesis, Drosophila larvae expressing HSF-GFP were dissected to obtain the salivary glands. These glands contain polytene chromosomes that allow for visualization of HSF binding onto the chromosome using confocal microscopy. The major binding site that is easily visualized is a doublet of HSF binding at the Hsp70 loci on the chromosome. Salivary glands at room temperature function as a non-heat shock control and exhibit no binding of HSF-GFP at the Hsp70 loci. Salivary glands heated to 37C for 10 minutes function as the positive control and exhibit the expected Hsp70 doublet from HSF-GFP binding of the DNA. We are testing variable concentrations of resveratrol to determine if it activates HSF-GFP binding of the DNA in salivary glands under non-heat shock conditions. Future experiments may examine if the HSF-GFP is transcriptionally active when cells are treated with resveratrol.

234V Same tissue, different responses: How do different cells in the *Drosophila* wing imaginal disc respond to ionizing radiation and contribute to tissue homeostasis? *Joyner Cruz*, Andreana Gomez, Iswar Hariharan University of California Berkeley

Tissues consist of highly organized cell populations which must mount coordinated responses to damaging stressors in order to maintain tissue viability. In the *Drosophila* wing imaginal disc, observations of coordinated homeostatic responses to diverse damaging events are abundant- however, the exact mechanisms of this coordination are not fully understood. Here we show that cells in the notum of the wing disc are resistant to ionizing radiation, and that they lose this resistance through developmental time. We also show that apoptosis in this region occurs independently of the JNK stress-signaling pathway, in contrast to other regions of the disc. Our results provide further evidence that different cell subpopulations in the wing disc, which are canonically considered to be of the same cell type, have strikingly heterogeneous responses to ionizing radiation and may contribute to total tissue response in specialized ways.

235C Characterization and functional analysis of diverse reactive Oxygen species produced during the immune response to bacterial infection. *Alva Duenas*, Caitlin Harris, Catherine Brennan Cal State University Fullerton

Reactive Oxygen Species (ROS) are a class of highly reactive Oxygen-containing ions and molecules that can be produced in cells by direct enzyme-mediated catalysis, as byproducts of mitochondrial respiration, and as secondary metabolites produced by the reaction of ROS with each other and other molecules in the cell. Identifying these short-lived molecules can be challenging due to their short-lived nature, as well as their similar chemistries and interactions with commonly used fluorescent ROS probes. In the immune response, ROS are produced in both fly and mammalian macrophages, where they play roles in both direct killing of engulfed microbes, as well as regulatory roles in macrophage activation and inflammation. We are dissecting the fly macrophage ROS response using a combination of genetic and biochemical tools, to better characterize ROS produced at different stages of the immune response, and to determine their distinct functions. We find that reactive Nitrogen species (RNS) produced by Nitric Oxide Synthase modulate the ROS response, and are responsible for some inflammatory processes previously ascribed to ROS.

236A Identification and characterisation of functionally distinct macrophage subpopulations in *Drosophila* *Martin Zeidler*[1], Jonathon Coates [1,2,3], Elliot Brooks[2], Amy Brittle[2], Emma Armitage[2], Iwan Evans[2] 1) The Bateson Centre, School of Biosciences, University of Sheffield, Sheffield, United Kingdom.; 2) Department of Infection, Immunity and Cardiovascular Disease and the Bateson Centre, University of Sheffield, Sheffield, United Kingdom.; 3) Current address: William Harvey Research Institute, QMUL, London, United Kingdom

Drosophila blood is dominated by a macrophage-like lineage of cells termed plasmatocytes which differentiate in two waves during embryonic and L3 larval development and are maintained throughout adult life. Throughout this time, plasmatocytes fulfil multiple roles, including wound responses, clearance of apoptotic debris and the provision of an innate immune response to infection. However, until very recently, the plasmatocyte lineage was thought to represent a homogeneous population – a stark contrast to vertebrate macrophages which assume a range of diverse functional and gene expression states.

Here we present our identification of a number of enhancer elements labelling plasmatocyte subpopulations, which we show to vary in abundance across development. We show that some of these subpopulations exhibit functional differences compared to the overall blood cell population, including more potent responses to wounds, altered apoptotic cell clearance and differential localisation / dynamics in pupae and adults. Genes located adjacent to enhancers showing altered plasmatocyte behaviour include Calnexin14D, which, when over expressed in all plasmatocytes, improves wound responses, causing the overall population to more closely resemble the marked subpopulation. These results suggest that differential gene expression not only labels, but also drives changes in subset behaviour. Finally, we show that exposure to increased levels of apoptotic cell death is sufficient to modulate the number of cells within certain subpopulations – further suggesting that subpopulations are dynamic and respond to environmental cues and stresses.

Taken together, our work demonstrates a degree of adaptive and functional macrophage heterogeneity in *Drosophila* that has not previously been described. It has identified mechanisms involved in subpopulation specification and function and provides a starting point for future studies into functional in vivo *Drosophila*macrophage heterogeneity.

237B Diptericin A protects flies from opportunistic gut infections in a sex dependent manner *Sarah Mullinax*, Robert Unckless University of Kansas

Living things must be able to balance their immune defenses to control potentially pathogenic microbes while also fostering beneficial ones. This is especially important when considering gut immunity. Most animals are constantly ingesting bacteria from their food and their gut immunity must be able to rid the animal of potentially harmful bacteria while maintaining beneficial bacteria. Several have shown that the humoral immune system influences the gut microbiome in *Drosophila*, but there has been much less investigation of the role of specific effector genotypes in shaping the microbiome and microbiome-related phenotypes. In this this study we focused on the *Drosophila* antimicrobial peptide Diptericin A. Diptericin A is especially important in the defense against a systemic infection by the Gram- bacteria *Providencia rettgeri* and the survival probability is highly dependent on the genotype of *diptericin A*. Diptericin A is expressed in the gut under normal rearing conditions. We tested the effect of the presence/absence and genotype of *diptericin A* in the gut after oral infection using gnotobiotically reared flies. We assessed the effect of mono- and poly-association with common gut bacteria including *Acetobacter* and *Lactobacillus* species as well as with *P. rettgeri.* We found remarkable sex-specific, microbe-specific and sex-by-microbe effects on bacterial load and fitness-related traits. Most markedly we found a mono-association with *Lactobacillus plantarum* greatly reduced the lifespan of female *diptericin A* null flies. This decrease in lifespan in *diptericin A* null flies was also observed in poly-associations that included *L. plantarum*. Surprisingly these results were not seen in male flies, lending support to the need to include both sexes in microbiome studies. Furthermore we are seeing a potential diptericin genotype effect when female flies are poly-associated with *L. plantarum* and *A. tropicalis* which is interesting in the context of there also being genotype effects after systemic infections. Overall this research characterizes the importance of having functional *diptericin A* in the gut for fly fitness.

238C Zika Virus infection in *Drosophila* brain activates host immune responses in a sex-dependent manner *Ghada Tafesh*, Ananda Kalukin, Ioannis Eleftherianos The George Washington University

With World Health Organization (WHO) scientists recently confirming an outbreak of Zika virus (ZIKV) in India, the characterization and understanding of the molecular mechanisms underlying immune responses against ZIKV have, once again, become urgent. ZIKV, a member of the *Flaviviridae* family, is transmitted to humans primarily by the infected *Aedes* mosquito species, causing neurologic complications that range from sensory neuropathy and seizures to congenital Zika syndrome (microcephaly) in infants born to mothers infected during pregnancy. Previous research presents compelling evidence that establishes the fruit fly *Drosophila melanogaster* as a reliable model for studying arboviruses, as many of the signaling pathways identified are evolutionarily conserved amongst insect species. Particularly, the large conservation between *Drosophila* and mosquitoes has paved the way for our study to provide novel insights into ZIKV pathogenesis and host antiviral immune function using a brain tumor *Drosophila* model. By examining gene expression in adult *Drosophila*, we show that ZIKV can replicate efficiently in the brain and activate various immune responses. More specifically, our results confirm that RNA interference (RNAi) plays a key role in limiting ZIKV replication in the brain, thus forming a potent antiviral defense in *Drosophila* and presenting a potentially powerful tool to combat the host brain tumor. Behavioral analysis also shows that ZIKV causes severe locomotor impairment in infected flies, a common phenotype of neurodegenerative diseases such as Zika. Most importantly, our observations show significantly higher ZIKV rates and effects in female flies than males, indicating possible differences in the rates of infection and susceptibility to the development of disease. These findings make it imperative to continue uncovering the specifics of the complex host-virus interactions and provide additional insights that can potentially be harnessed for the control of different viral pathogens and define the full spectrum of antiviral immunity across various hosts.

239A Short-term feeding on high sugar increases susceptibility to infection *Andrea Darby*[1], Destiny Okoro[1], Brian Lazzaro[1,2] 1) Cornell University; 2) Cornell Institute of Host-Microbe Interactions and Disease

An organism's diet is a critical factor for its ability to survive an infection, and overnutrition of dietary sugar in particular has been demonstrated to increase susceptibility to infection across animal models and humans. Adult *Drosophila melanogaster* reared on high sugar diets experience higher bacterial burdens and higher mortality post infection, although the genetic and physiological mechanisms that lead to this outcome are not well understood. Prior studies that have investigated the impact of high dietary sugar on survival of infection have been performed using flies that that fed on high-sugar diets throughout their entire life, thus making it difficult to distinguish developmental consequences from acute metabolic effects on immunity. We hypothesized that even transient exposure to high-sugar diet might cause metabolic dysregulation with adverse consequence for immune function. To test this hypothesis, we reared *D. melanogaster* on a standard 4% (w/v) sucrose diet, then transferred them to one of six experimental diets varying from 0% - 24% sucrose upon eclosion. After 3-5 days of feeding, we assayed their infection survival over five days against systemic infection with two Gram-negative bacteria, *Providencia rettgeri* and *Serratia marcescens*, and one Gram-positive bacteria, *Enterococcus faecalis*. I found that even three days of exposure to elevated sugar in the diet was sufficient to significantly reduce the probability of surviving infection. However, this effect was specific to the Gram-negative bacteria assayed, suggesting that the immediate metabolic effects of high sugar may have disproportionate impact on the immune pathways that are required for combating Gram-negative infection. In ongoing work, we are testing infection survival in other Gram-positive bacteria, like *Lactococcus lactis*, to determine whether the sugar content affect is specific to Gram-negative bacteria. Additionally, we are testing whether elevated dietary sucrose specifically impairs IMD pathway signaling, and the extent to which different pathogens may themselves have different sensitivities to the excess sugar within their host. These results are foundational for further understanding of the genetic and physiological mechanisms by which high sugar impacts infection survival.

240B Phagocytosis-dependent activation of Nrf2 strengthens the macrophage inflammatory response whilst limiting immune senescence and systemic tissue damage. *Giuliana Clemente*, Helen Weavers University Of Bristol

The respiratory burst is a powerful weapon utilized by leukocytes to protect the host from dangerous pathogens and degrade cellular debris. Nevertheless, Reactive Oxygen Species (ROS) are exceptionally reactive molecules that could cause serious bystander damage to both, the phagocyte itself and the surrounding environment. Therefore, to sustain full functionality under these hostile conditions, immune cells must trigger a complex and robust self-protection program. This cytoprotective system must be carefully orchestrated to ensure leukocytes limit the undesirable side-effects of the constant oxidative challenge, while allowing low levels of ROS for crucial cellular signalling.
Here, we discuss the molecular nature of these self-protective strategies, dissecting *in vivo* both their precise mechanism of activation and physiological relevance. We provide evidence that *Drosophila* macrophages robustly activate the master redox-sensitive transcription factor Nrf2 upon apoptotic corpse engulfment and this activation is driven by calcium and PI3K-dependent release of ROS by NOX oxidases at the phagosome. We find that this Nrf2-mediated antioxidant response is vital to promote *Drosophila* macrophage tolerance to oxidative stress during normal homeostatic behaviour, preserving efficient basal motility and allowing a robust and timely detection of epithelial wounds. Moreover, by reducing the oxidative burden, Nrf2 not only delays the onset of immune-senescence but supports host longevity by

limiting collateral damage to surrounding tissues. Overall, we find that by preserving a healthy immune system, leukocyte Nrf2 plays important paracrine effects by delaying premature systemic aging and improving organismal lifespan. We propose that future therapeutic interventions aimed to boost immune self-protection may be beneficial to delay immune aging and alleviate correlated morbidities.

241C Identifying Candidate Genes and Genetic Networks that Influence the Age-specific Ability to Clear an Infection Using Genome Wide Association Tests (GWAs) *Shonda Campbell*, Jeff Leips University of Maryland Baltimore County

The innate immune response is an evolutionarily conserved process that is essential for host survival in all multicellular organisms. As individuals age, immune functions begin to decline, or immunosenesce, reducing one's ability to fight infections, posing a serious risk to human health. The way that age affects the immune response can vary greatly among individuals, and although this variation has a significant heritable component, the genes responsible for this variation are not known. Previous data using 12 inbred *Drosophila melanogaster* lines from the *Drosophila* Genetic Reference Panel (DGRP) found that the genes implicated in clearing an infection were different between young and aged flies. The goal of this project is to identify genes that regulate age-specific immune responses to better understand the mechanisms that give rise to immunosenescence. Currently, standard bacterial clearance assays and genome wide association tests (GWAs) are being performed using the 192 DGRP lines available. To assess the ability of each line to clear an infection, one and five week old virgin females are injected with 18.4nL of an *E. coli* solution at an OD_{600} of 1.0+ 0.01. After 24-hours, the surviving flies are individually homogenized and plated on streptomycin LB/agar plates. The colony forming units per milliliter (CFU/mL) represents the phenotype of that individual and reflects the remaining bacteria in the fly. A GWAS will then be performed at both ages to identify candidate genes involved in clearance across ages. Those candidate genes will be the focus of future studies where the results could lead to improved therapeutic treatments in an aging population, providing age-appropriate drug targets to restore the immune function.

242A Modified binding site of IDGF proteins is important for their function Vaclav Broz[1], Houda Ouns Maaroufi[1,2], Michal Zurovec[1,2], *Lucie Kucerova*[1] 1) Biology Centre of the Czech Academy of Sciences, Institute of Entomology, Ceske Budejovice, Czechia; 2) University of South Bohemia, Faculty of Science, Ceske Budejovice, Czechia

IDGFs (Imaginal Dish Growth Factors) are a family of chitinase-like proteins secreted into the insect hemolymph from the fat body and hemocytes, they also represent important component secreted from salivary glands and silk glands of insects. IDGFs lost the hydrolytic activity of true chitinases but they are still able to bind carbohydrate moieties. They are implicated in multiple functions, including extracellular matrix reorganization, cell growth stimulation, wound healing, insect hemolymph clotting, antimicrobial activity, detoxification and also insect silk spinning. With its crystal structure determined, *Drosophila* IDGF2 is the best characterized member of the IDGF family. IDGF2 possess a unique shaped carbohydrate binding domain which is different from chitinases and other chitinase-like proteins.
We conducted a glycan array screen to find the binding partner of IDGF2. We established a functional *in vivo* assay based on the ability of IDGF2 to induce genes involved in innate immunity and inflammation. We compared the induction of wild-type and binding domain mutant forms of IDGF2. Moreover, we also examined the mutant protein distribution in tissue.
In the present study, we have identified Galα1-3(Fucα1-2)Galβ1 carbohydrate moiety as the preferred binding partner of IDGF2. Unlike other known chitinase-like proteins, IDGF2 does not bind to N-acetylglucosamine. Furthermore, we proved that the function of IDGF2 depends on its binding ability.
Our work brings new insights about insect chitinase-like proteins binding abilities, which are crucial for their function as modulators of host response in insects as well as mammals.

243B Phagocytic defects lead to or exacerbate neurodegeneration through increased immune signaling *Guangmei Liu*[1], Johnny Elguero[1], Katie Tiemeyer[1], Heena Gandevia[1], Iker Etchegaray[1], Mel Feany[2], Kim McCall[1] 1) Department of Biology, Boston University, Boston, MA; 2) Department of Pathology, Harvard Medical School, Brigham and Women›s Hospital, Boston, MA

In nervous system development, as well as in disease and injury, neurons die through programmed cell death, leaving behind cell corpses which must be removed. The clearance of these corpses is accomplished through phagocytosis. In the nervous system, glial cells act as phagocytes, engulfing dead neurons and debris to ensure proper morphology and tissue homeostasis. Glial phagocytosis has been implicated in several neurological diseases. In humans, increased numbers of phagocytic glia are observed in conditions like Alzheimer's disease, Parkinson's disease, and traumatic brain injury. In vitro, glia have been shown to clear protein aggregates like those found in neurodegenerative disease. Moreover, variants of genes implicated in glial phagocytosis have been identified as risk factors for neurodegenerative diseases. However, how phagocytosis defects might cause or worsen neurodegeneration remains unknown. We are using the *Drosophila melanogaster*, whose complex nervous system harbors phagocytic glia analogous to those in humans, to tackle this question. Our lab previously found that mutant flies lacking the phagocytic receptor Draper show an accumulation of neuronal cell corpses, which result from developmental programmed cell death and persist throughout the organism's life. And flies lacking glial Draper display age-dependent neurodegeneration.

To determine how phagocytic defects lead to neurodegeneration in the *draper* mutant, we investigated the hypothesis that persisting cell corpses in the brain lead to chronic increased immunity, resulting in neurodegeneration. We measured activation of the immune pathway Imd in aging *draper* mutants and found that the antimicrobial peptide *attacin* A is highly overexpressed in fat body. We then suppressed the Imd pathway by knocking down *Relish* in glia and fat body in *draper* mutants and found that neurodegeneration was reduced, indicating that immune activation promotes the neurodegeneration in *draper* mutants. Taken together, these findings indicate that phagocytic defects lead to or exacerbate neurodegeneration through increased immune signaling, both systemically and in the brain. To define the causality of neurodegeneration in *draper* mutants, we are testing two hypotheses that the neurodegenerative phenotype results from persisting corpses or from defective phagocytic function of glia. We are comparing the severity of neurodegeneration in aging flies with either only persisting corpses or only defective phagocytic function. To further define the consequences of neurodegeneration caused by knocking down *draper* in fly glia, we are charactering the cellular identities of dead neurons in these aging flies and examining related behavioral deficits such as locomotion activity and learning and memory function.

244C Peroxisomes regulate the Imd amyloid fibril formation and subsequent Relish signaling pathway *Yizhu Mu*[1,3], Anni Kleino[2], Neal silverman[2], Francesca Dicara[1,3] 1) Dalhousie university, Halifax, NS, Canada; 2) University of Massachusetts Medical School, Worcester, MA, USA; 3) IWK heath center, Halifax, NS, Canada

The Imd pathway plays a pivotal role in the Drosophila defense response against bacterial infection. Gram-negative bacterial infection activates Imd protein which promotes the activation of the NF-κB homolog Relish for the robust antimicrobial peptide gene expression. Although Imd activation is finely regulated, our knowledge of the cellular mechanisms modulating the Imd pathway is still in its infancy. Understanding of the Imd pathway is further complicated by the involvement of Imd protein in the formation and resolution of amyloid-like structures which are indispensable for the activation of the Imd pathway. Peroxisomes are essential lipid metabolic organelles known to play an important role in innate immunity through regulation of cellular lipids milieu in the activated immune cells. Moreover, it is known that defects in peroxisome metabolism inhibits the activation of the Imd pathway. Therefore, we hypothesize that changes in the lipid milieu at the plasma membrane might contribute the Imd amyloid fibril formation and subsequent signaling. Here we demonstrated that peroxisomes regulate Imd amyloid-like structure formation and the activation of the Imd signaling. Using immunofluorescence experiments of *Drosophila* S2 cells, we found that Gram-negative bacterial infection results in the robust amyloid formation in wild type (WT) S2 cells whereas in the absence of functional peroxisomes (peroxisome depleted cells) amyloid structures are not detected. Furthermore, bacterial infection results in the formation of distinct Imd punctate structures that co-localize with amyloid structure in the WT S2 cells suggesting the activation of the Imd pathway. In contrast, peroxisome deficient S2 cells show impairment in the formation of distinct Imd punctate structures in response to bacterial infection. Furthermore, lipids such as PIP2 and PA (whose biosynthesis are known to require peroxisomes) were found to interact with IMD protein *in vitro*. Altogether, our initial study shows that peroxisomes play a functional role in the activation of the Imd pathway by regulating IMD amyloid structures formation.

245A Identification of Enhancers of the *Drosophila* Innate Immune System *Lianne Cohen*, Zeba Wunderlich Boston University, Boston MA

An organism's ability to launch an appropriate and robust response to environmental stimuli is critical for its survival. Infection from a variety of microorganisms is a common and potentially lethal threat that host organisms combat by initiating an immune response. Much of this response is controlled by 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. Without knowledge of these regulatory elements, our understanding of how immune signal pathways interact at the *cis*-regulatory level and the grammar needed for this type of gene expression is limited. To identify immune responsive enhancers, we stimulated the major immune pathways, Toll and IMD, both individually and together in S2 cells and measured enhancer activity via STARR-seq (Self transcribing active regulatory-region sequencing). This activity-based assay identifies sequences that can drive their own expression upon activation of immune signaling pathways in a high-throughput manner. A large-scale analysis of immune enhancers permits us to characterize the regulatory grammar found in these sequences, as well as unknown immune TFBS. Additionally, by stimulating two immune pathways in this experiment we can investigate the role enhancers play in producing synergetic gene expression. The identification of immune enhancers will provide insights into how animals use regulatory sequences control their gene expression response to a rapidly changing environment and thus survive infection from pathogens.

246B Title: Exploring transcriptional signatures of Anti-Microbial Peptides early in infection to predict infection outcomes *Radhika R*[1], Brian Lazzaro[1,2] 1) Cornell University; 2) Cornell Institute of Host-Microbe Interactions and Disease (CIHMID)

Production of Anti-Microbial Peptides (AMP) is an integral part of the anti-bacterial infection response in insects. Controlled primarily by the Toll and Imd signaling pathways, a wide array of AMP genes are upregulated within four to six hours of hosts encountering bacterial pathogens. A model of infection progression developed by Duneau *et al*. 2017 suggests that this early phase of infection response, lasting up to about twelve hours, is very important for effective pathogen control. Hosts that survive infection do so by restricting pathogen load to a low, sustained residual burden that they retain for the rest of their life, while those that allow pathogens to grow unchecked succumb to infection. In this study, using *Drosophila melanogaster* as our model system, we test whether differences in AMP induction kinetics in the early phase of infection predict how well hosts curtail pathogen growth and thereby survive infections. Our hypothesis is that faster upregulation of the AMP(s) will correlate with increased probability of host survival and lower residual bacterial loads among surviving hosts. We first systematically tested which AMP(s) are the most important for controlling infection by each of four different bacteria using a panel of mutants that are deficient for different combinations of AMP genes. We then measured ten lines from the Drosophila Genome Reference Panel (DGRP) for their ability to survive infection with the four bacteria. In ongoing studies, we are testing whether the kinetics of AMP upregulation predict the probability of host survival of infection, specifically predicting that the strongest correlation will be seen with the expression of AMPs that are disproportionately important for controlling an infection by a given bacteria while other co-regulated AMPs may correlate more weakly. If the data support this hypothesis, that would provide key evidence that the rapid induction of AMP gene expression is essential for control of infection.

247C Metchnikowin alleles are associated with both immune and life history phenotypes *Jessamyn Perlmutter*[1], Joanne Chapman[2], Robert Unckless[1] 1) Department of Molecular Biosciences, University of Kansas, Lawrence, KS; 2) Enteric, Environmental and Food Virology Laboratory, Institute of Environmental and Scientific Research, New Zealand

Antimicrobial peptides (AMPs) are at the interface of interactions between hosts and microbes and are therefore expected to be fast evolving to keep pace with pathogens. However, production of these peptides is also costly to animals and the host must balance its own energetic and antimicrobial interests. Previous work identified distinct peptide alleles that are repeatedly maintained not only within populations of *Drosophila melanogaster*, but also across at least three other fly species. The maintenance of these polymorphisms across both geography and more than 10 million years of divergence, coupled with evidence showing balancing selection on AMPs in flies, suggest there is a distinct functional importance to each allele. Is each allele more effective against specific pathogens (specificity hypothesis), or is one allele more effective generally, but also more costly to produce (autoimmune hypothesis)? One AMP, Metchikowin (Mtk), has a single residue that segregates as either proline (P) or arginine (R) in populations of four *Drosophila* species. To assess the functional difference between the alleles, we created *D. melanogaster* lines with the P allele, the R allele, or a Mtk null mutation using CRISPR/Cas9 genome editing. Here, we report results from experiments assessing the specificity versus autoimmune hypotheses using these lines. Testing of the flies against a repertoire of bacteria and fungi demonstrated differences in survival rates across allele or null mutation lines with some pathogens. In addition, measurements of various life history traits in these lines reveal differences in longevity in adulthood, suggesting differential fitness costs to producing the alleles. In summary, there are various measurable functional differences between Mtk alleles in *Drosophila melanogaster*, providing several lines of support for adaptive maintenance of Mtk polymorphisms in contrast with expectations of rapid AMP evolution.

248A JAK/STAT mediated metabolic reprogramming during immune response *Ellen McMullen*, Lukas Strych, Tomas Dolezal University of South Bohemia in České Budějovice

JAK/STAT is a highly conserved pathway, which plays a key role in immune response. In the case of Drosophila larvae, JAK/STAT signaling is involved in differentiation of hemocytes into lamellocyte to fight against parasitoid wasp infection (*Leptipilina boulardi*). This, in part, is mediated by the secretion of cytokines Upd2 and Upd3 from hemocytes, which activates JAK/STAT signaling in skeletal muscles; required for the efficient encapsulation and melanization of the wasp egg. Deletion of Upd2 and Upd3 leads to significant reduction in lamellocyte number, and therefore efficient immune response.

In times of food scarcity, or high metabolic demand tissues compete for the nutrients available. Parasitoid wasp infection leads to a redistribution of nutrients from surrounding tissues, such as muscles and fat body, to provide sufficient energy for immune response. This supports the 'selfish' or 'privileged' immune system hypothesis, as the immune cells are prioritized over other tissues to aid the survival of the animal. Reduction in carbohydrate uptake in peripheral tissues during infection is thought to be mediated by suppression of insulin signaling (IS) in these non-immune organs. Our findings show lower levels of pAkt in the larval fat body and skeletal muscles during infection, indicating IS suppression. We propose the reduction in IS observed is a result of Insulin resistance (IR) in these tissues. Furthermore, we suggest this IR occurs via Upd2 and Upd3 mediated activation of JAK/STAT in muscles leading to a systemic metabolic reprogramming during infection.

249B The Role of Professional Phagocytes during Cell Death in the Ovary of *Drosophila melanogaster* *Alexandra Chasse*, Shruthi Bandyadka, Max Werthemier, Kim McCall Boston University, Boston, MA

Regulated cell death is a key process necessary for maintenance of homeostasis and removing toxic cells from the organism and involves signaling responses among several cell types that are not well understood. Apoptosis is the best understood type of regulated cell death and once cells begin apoptosis, they produce multiple signaling cues to entice phagocytes. Macrophages are the professional phagocytes (PPs) that clear apoptotic cells in many tissues and organs, however other cells, such as tissue resident epithelial cells, act as non-professional phagocytes (NPPs). While there has been extensive research on mechanisms used to engulf dying cells for both PPs and NPPs, there is little research done to identify mechanisms used for communication. The *Drosophila* ovary is a powerful model for cell death mechanisms despite being "immune-privileged" meaning that the ovary is protected from an uncontrolled immune response by denying hemocytes access to the organ. However small numbers of hemocytes have been detected in the ovary and previous work has shown that the cytokine Unpaired 3 (Upd3, IL-6 like) is highly expressed in NPP Follicle Cells (FCs) during engulfment of apoptotic Nurse Cells (NCs) but is not required for engulfment. Another pro-inflammatory cytokine, Eiger (TNF-like), was shown to be required for cell clearance but it was not determined whether it is required by the FCs or the NCs. Further, the fate of follicle cells after engulfing nurse cells is still unknown. We are characterizing the response of hemocytes when cell death is induced in the ovary as well as investigating the signaling pathways used by professional and non-professional phagocytes to maintain homeostasis during the complete removal of dying cells.

250C The role and regulation of metabolic enzymes *astray* and *Nmdmc* during infection *Krista Grimes*[1], Esteban Beckwith[1,2], Marc Dionne[1] 1) MRC Centre for Molecular Bacteriology and Infection, and Department of Life Sciences, Imperial College London; 2) Instituto de Fisiología, Biología Molecular y Neurociencias (IFIBYNE), UBA-CONICET, Buenos Aires

Immunity and metabolism are closely interconnected processes. Mounting an immune response is energetically costly, and therefore relies on metabolism adapting to provide the necessary energy and raw materials. The aim of this work was to further characterise the metabolic changes that occur in *Drosophila* during bacterial infection. Using Targeted DamID, we found that two metabolic genes with linked roles in amino acid metabolism, *astray* and *Nmdmc,* are upregulated in the fat body during infection. *astray* is a phosphoserine phosphatase that catalyses the final reaction in the *de novo* production of serine from glycolytic intermediates. Serine can be converted into glycine using a tetrahydrofolate cofactor, which is produced by a cycle of reactions involving *Nmdmc.* We found that in flies infected with Gram positive bacteria, the upregulation of *astray* and *Nmdmc* is dependent on both Dif and FOXO, key components of the Toll and insulin signalling pathways, which may act in a co-ordinated manner. Further, fat body knockdown of the transcription factor MEF2 leads to an increase in the expression of *astray* and *Nmdmc* in uninfected flies, suggesting that MEF2 may contribute to the repression of both genes in the absence of infection. We next sought to establish whether *astray* and *Nmdmc* play functional roles in supporting the immune response. Fat body specific *astray* knockdown results in a significant survival defect during *Staphylococcus aureus* infection, whilst bacterial numbers remain unchanged, indicating that *astray* contributes to host tolerance. In contrast, knockdown of *Nmdmc* in the fat body leads to a modest survival increase. Together, these data demonstrate that metabolism is transcriptionally fine-tuned in a manner that has complex impacts on the course and outcome of infection and indicate that regulation of amino acid metabolism via FOXO is critical to support host health during immune responses.

251A Endocrine regulation of metabolism and immunity in response to commensal and pathogenic bacteria *Scott Keith*[1,2], Brian Lazzaro[1,2], Brooke McCartney[3] 1) Department of Entomology, Cornell University, Ithaca, NY; 2) Cornell Institute of Host-Microbe Interactions and Disease, Cornell University, Ithaca, NY; 3) Department of Biological Sciences, Carnegie Mellon University, Pittsburgh, PA

Animals are continuously exposed to diverse populations of environmental microbes ranging from beneficial bacteria to harmful pathogens and must rapidly adjust energy metabolism and immune reactions in response. Circulating hormones can modulate the balance between interrelated physiological processes through effects on gene expression and cell biology. In *Drosophila*, ecdysone and insulin signaling sustain metabolic homeostasis, developmental growth, and immunity in the context of both commensal microbiota association and infection with pathogens. However we know little about the mechanisms of this regulation, including the extent to which these hormones facilitate immuno-metabolic crosstalk and how interactions with microbes shape that crosstalk. We have identified the neuronal synaptic plasticity factor Arc1, which is homologous to mammalian Arc/Arg3.1 proteins, as a novel host gene that supports larval metabolism and growth in response to microbiota perturbation. We found that Arc1 can function in the insulin-producing cells of the larval brain and the ecdysone-synthesizing prothoracic gland to support development in germ-free (GF) Drosophila, and that hallmarks of insulin and ecdysone signaling were perturbed in GF *Arc1* mutants. We additionally discovered that flies lacking Arc1 exhibit altered antimicrobial peptide induction levels, increased microbial loads, and increased mortality after systemic infection with the bacterial pathogens *Erwinia* (*Pectinobacter*) *carotovora* and *Providencia rettgeri*. These data reveal an unexpected new role for Arc proteins, which have been previously studied exclusively in the context of the nervous system. Future work will test whether Arc1 regulation of ecdysone and insulin signaling affects immune responses to systemic infection. Ongoing research will directly investigate how the interplay between ecdysone and insulin signaling in the fat body regulates IMD

pathway function and restructures energy metabolism to ensure survival of infection, potentially at the cost of resistance to other stressors like nutritional challenge. Through this work, we will better understand the mechanistic principles of hormone-mediated metabolic and immune system regulation in the context of host-microbe interactions.

252B Domestication of a phage-encoded DNAse I by *Drosophila* *Rebecca Tarnopol*, Jaden Ha, Susan Bernstein, Kirsten Verster, Noah Whiteman University of California, Berkeley

Advances in genomics have illuminated a potential role of horizontal gene transfer (HGT) as a source of evolutionary novelty in animals. We discovered the horizontal transfer of the *cytolethal distending toxin B* (*cdtB*) gene from bacteriophage (phage) donors into the nuclear genomes of several insects, including drosophilid flies. *cdtB* is a major virulence factor found in Proteobacteria and Actinobacteria that encodes a DNAse I-type nuclease. CdtB homologs encoded by bacterial pathogens of mammals typically induce cell cycle arrest and apoptosis of eukaryotic host cells. In secondary endosymbiotic bacteria of pea aphids (*Ca*. Hamiltonella defensa), phage-encoded *cdtB* may protect the insect host against parasitoid wasp attack. We hypothesize that HGT-derived *cdtB* is playing a similar protective role in the *Drosophila* lineages that harbor it. How insects are able to deploy HGT-derived *cdtB* to potentially kill parasitoids without harming their own cells remains unclear. Addressing this outstanding question is essential to understanding how a virulence gene that targets eukaryotic cells became functionalized within a eukaryotic genome. We hypothesize that drosophilid hosts may mitigate CdtB toxicity using a number of strategies, such as limiting its expression to particular tissues, or that the protein has evolved to not target the host genome (i.e., through modified substrate specificity or interactions with native host proteins). Here, we used the UAS-GAL4 system to test toxicity of *cdtB* expression in *Drosophila melanogaster*, which does not natively encode *cdtB*. We conducted in vitro nuclease assays to determine the specificity of drosophilid CdtB towards methylated vs. unmethylated DNA. We further investigated mechanisms by which drosophilid flies may evade CdtB toxicity by expressing the toxin in yeast. Overall, our work illuminates how prokaryotic genes whose products evolved to kill eukaryotic cells are integrated into animal gene networks.

253C ShKT-domain-containing protein from parasitic nematode is toxic to *Drosophila melanogaster* *Aklima khanam Lima*, Harpal Dhillon, Adler Dillman University of California Riverside

Entomopathogenic nematodes (EPNs) are insect-parasitic nematodes which kill insects quickly on infection. EPNs from the genus *Steinernema* release excretory/secretory proteins (ESPs) into the insect host at the early stages of infection and these ESPs are toxic to insects. These proteins actively participate in tissue damage and immunomodulation of the host during infection. One of these proteins is an ShKT-domain-containing protein. ShKT-domain-containing proteins are named after the venom of sea anemone *Stichodactyla helianthus*. The ShKT domain is found in diverse groups of animal venoms and forms a large group of family called the ShK superfamily. They are known as a potent K^+ channel blocker and perform many critical roles in animal venoms e.g., immunomodulator, neurotoxic, paralytic, hemolytic, etc. Our goal is to study the function of the ShKT protein from *S. carpocapsae* nematode and characterize its role in host-parasite interactions. We generated a recombinant version of ShKT protein using yeast expression system. To characterize the activity of the protein, we used the fruit fly (*Drosophila melanogaster)* model system. We injected 100 ng of purified ShKT protein into fruit flies and observed ~50% mortality. In addition to mortality, we evaluated additional health metrics of the fruit flies using behavioral assays (negative geotaxis and chill coma recovery). We found that the health of fruit flies was significantly affected by the ShKT protein.

254A Nematode secreted PLA_2 displays toxicity and immunosuppression in Drosophila melanogaster *Ogadinma Okakpu*, Sophia Parks, Harpal Dhillon, Adler Dillman University of Califorina Riverside

Parasitic nematodes are an ongoing problem for human health and agriculture. Key to their success in infection is the ability to evade or suppress host immunity. This immunomodulatory ability is likely due to the release of hundreds of excretory/secretory proteins (ESPs) upon infection. While ESPs have been shown to display immunosuppressive effects on a host, there are still gaps in understanding about the molecular interactions between individual proteins released and host immunity. Our lab has recently identified a secreted phospholipase A_2 ($sPLA_2$) released from the entomopathogenic nematode (EPN) Steinernema carpocapsae, which has immunosuppressive and toxic capabilities in insects. Immunosuppression by Sc-$sPLA_2$ is characterized by its ability to suppress the toll pathway and phagocytosis which lead to a decrease in survival against bacterial infections, and an increase in pathogenic bacterial growth in an infection model. Toxicity by Sc-$sPLA_2$ is characterized by increased mortality, with the severity being both dose and time dependent. EPNs have homology to vertebrate-parasitic nematodes, and thus can be used as model systems for understanding how nematode ESPs alter host immunity. $sPLA_2$ enzymes specifically, are known to be modulators of host immunity via lipid signaling. Understanding how Sc-$sPLA_2$ suppresses D. melanogaster host immunity will help elucidate EPN immunomodulation at the molecular level and may increase our understanding of the role of lipid signaling in insect immunity, which is an understudied aspect of the insect immune response.

255B ***Drosophila melanogaster*** **containing a galbut virus endogenous viral element are resistant to infection** *Ali Brehm*, Mark Stenglein Colorado State University, Fort Collins, CO

Galbut virus is a double-stranded RNA virus in the family *Partitiviridae* that is found in 100% of *D. melanogaster* populations but only in ~60% of individuals within those populations. Galbut virus infection produces no obvious phenotype, however, a small fitness cost could be sufficient to select for resistance, and multiple lines of evidence support the hypothesis that some flies are resistant to infection. Endogenous viral elements (EVE) are integrated viral sequences that represent potential mechanisms of viral resistance. Recently, a galbut virus EVE has been identified in European *D. melanogaster* populations. I hypothesized that the galbut virus EVE might confer resistance to galbut virus infection. To test this hypothesis, I created a line of flies homozygous for the EVE by crossing an inbred line containing the EVE, (Global Diversity Line N14) and a robust inbred line from the Drosophila Genetic Reference Panel (DGRP strain 517). To test whether flies with the EVE can become infected, I microinjected galbut virus EVE+ individuals and found that only a small frequency (less than 1%) of offspring of injected parents were infected. To test whether the EVE is responsible for resistance, I am using CRISPR/Cas9 to remove the EVE. By removing the EVE, experiments can be done in parallel with EVE+ and non-EVE flies that are identical otherwise to test whether the EVE is actually conferring resistance.

256C **Age-dependent antiviral immunity in** ***Wolbachia*****-infected** ***Drosophila melanogaster*** *Brian Kmiecik*, Casey Goltz, Lakbira Sheffield, Stanislava Chtarbanova The University of Alabama, Tuscaloosa, AL

Wolbachia (*Wolb*) is an obligate, maternally-transmitted, intracellular bacterium known to infect ~50% of all insect species on Earth, including *Drosophila*. One aspect of *Wolbachia-Drosophila* interactions is a phenomenon called "pathogen blocking," which corresponds to *Wolbachia*-mediated protection of *Drosophila* against pathogenic viral infections. The genetic and molecular mechanisms underlying this protection are not fully understood and are thus an ongoing area of investigation. Moreover, a gap in knowledge exists about the nature of the *Wolbachia-Drosophila* relationship as a function of host age. This is important to address as the aging process itself affects host physiology, including immunity to viral infections.
We have previously shown that aging impairs the ability of *Drosophila* to withstand the pathological consequences of infection (the viral 'tolerance' mechanism) with the RNA-containing Flock House Virus (FHV). Here, using 149 (66 *Wolb*-free and 83 *Wolb*-positive) lines from the *Drosophila* Genetic Reference Panel (DGRP), we demonstrate that the age-dependent survival of FHV infection is a continuous trait, and is significantly modulated by the presence of *Wolb* independently of underlying lifespan. For several of the *Wolb*-positive DGRP lines, we established *Wolb*-free stocks using standard tetracycline treatment. Our analysis of one line so far, DGRP320, indicates that age-dependent decrease in survival of FHV infection observed in *Wolb*-free flies is suppressed when *Wolb* is present in the stock. Using RT-qPCR, we show no significant changes in *Wolb* 16S gene expression, nor in FHV load between young and aged, *Wolb*-free and *Wolb*-positive hosts. Our results indicate that *Wolb* possibly modulates disease tolerance to FHV with aging, independently of *Wolb* load.

257A **The Evolutionary Genetic Basis of Bacterial-Mediated Embryonic Lethality** Dylan Shropshire[1], *Mahip Kalra*[2], Seth Bordenstein[2] 1) University of Montana; 2) Vanderbilt University

Wolbachia are maternally-transmitted, intracellular bacteria that occur in approximately half of arthropod species worldwide. They can spread rapidly through host populations via the cytoplasmic incompatibility (CI) drive system. CI causes embryonic death when infected males mate with uninfected females, but offspring of infected females are rescued. Two proteins, CifA and CifB, underlie the genetic basis of CI and rescue, but how amino acid sites across these proteins contribute to CI and/or rescue remain unknown. Here, we employed evolution-guided, substitution mutagenesis on conserved amino acids to understand their relative contributions to CI and rescue. We report that amino acids in CifA's N-terminal unannotated region and annotated catalase-related domain are important for both complete CI and rescue, whereas C-terminal residues in CifA's putative domain of unknown function are solely important for CI. Moreover, conserved CifB amino acids in the predicted nucleases, peptidase, and unannotated regions are essential for CI. Taken together, these findings indicate that (i) all CifA amino acids determined to be crucial in rescue are correspondingly crucial in CI, (ii) an additional set of CifA amino acids are uniquely important in CI, and (iii) CifB amino acids across the protein, rather than in one particular domain, are all crucial for CI.

258B **Microbial Influence on** ***Drosophila sechellia*** **Fitness on Octanoic Acid** *Jake Erley*, Zachary Drum, Caroline Pitton, Joseph Coolon Wesleyan University

The fruit fly Drosophila sechellia has evolved to become a dietary specialist for the fruit of Morinda citrifolia, also known as noni. This specialization is unexpected because the fruit contains toxic volatile compounds, namely octanoic acid (OA) that kills other Drosophila species. Our prior research found that adult D. sechellia flies exposed to OA had widespread down-regulation of genes with immune system functions. This suggests possible interaction between the D. sechellia microbial community and toxin resistance. Previous studies in insects have demonstrated important roles of both gut

microbes and endosymbionts like Wolbachia on organismal traits including toxin resistance. In order to determine if gut microbes and/or Wolbachia contribute to D. sechellia OA resistance we made three fly lines: D. sechellia with normal microbe assemblage (gut microbes and Wolbachia), D. sechellia with normal gut microbes but no Wolbachia and D. sechellia maintained on antibiotics that have very few if any associated microbes. Ongoing experiments are testing OA resistance in these lines, identifying genome-wide gene expression differences among the lines with different microbe assemblages and 16S metagenomics analysis of the microbial community present in each of the different lines generated for this study.

259C ***Kismet*** **affects gut biomechanics, the gut microbiome, and gut-brain axis in** ***Drosophila melanogaster*** *Chloe Welch*[1], Angelo Niosi[1], Henry Vo[1], Punithavathi Sundar[2], Aliyah Penn[1], Jeffrey Cavanaugh[1], Prince Yadav[1], Wendy Lee, PhD[2], Mikkel Herholdt Jensen, PhD[1], Eliza J. Morris, PhD[1], Kimberly Mulligan, PhD[1] 1) California State University, Sacramento; 2) San Jose State University

The gut microbiome may contribute to the pathophysiology of neurodevelopmental disorders (NDDs), yet it is unclear how NDD risk genes affect gut physiology in a manner that may alter bacterial colonization. We addressed this question using *Drosophila* melanogaster with a mutation in *kismet (kis),* the ortholog of the human autism risk gene *Chromodomain Helicase DNA Binding Protein 8 (CHD8).* We used *kis* mutant flies to examine gut biomechanics, gut microbiota, and to explore the connection between gut microbiota and behavior. To quantify changes in gut tissue mechanics, we affixed whole guts between two clips mounted on a high-precision force transducer and length controller, capable of measuring forces to micro-Newton precision. Our measurements revealed significant changes in the mechanics of *kis* mutant guts compared to wild-type, in terms of elasticity, strain stiffening, and ultimate tensile strength. To characterize the gut microbiomes, we used 16S metagenomic sequencing and found that loss of *kis* profoundly impacted the abundance of many bacterial taxa in the midgut. To investigate the putative connection between the gut microbiome and behavior, we treated *kis* mutant and control flies with an antibiotic and then evaluated courtship behavior. Depletion of the gut microbiome rescued courtship defects of *kis* mutant flies, indicating a connection between the mutant gut microbiome and behavior. In striking contrast, depletion of the gut microbiome in the control strain induced courtship defects. This result demonstrates that antibiotic treatment can have either positive or negative impacts on behavior, depending on the status of gut dysbiosis prior to treatment.

260A **The relationship between natural diet, microbiome, and life history in** ***Drosophila melanogaster*** *Brittany Burnside*, Sarah Gottfredson Brigham Young University

My project focuses on the relationship between natural diets, the microbiome, and *Drosophila melanogaster* life history traits. I am studying the intersection between these traits in order to better understand how dietary impacts on host microbiota alters *D. melanogaster* life history.
As a starting point, I have measured the development rate and starvation resistance of a *D. melanogaster* CantonS stock with an unmanipulated microbiota ('conventional') when reared on 22 different, autoclaved diets (12 vegetables and 8 fruits). Eclosion time for each fly vial was measured every 6 hours until eclosion of all eggs was complete. Then, 5–10 day-old female flies were transferred in groups of 10 into vials containing 1% agarose, and their survival was measured every 4 hours until all flies in a vial were dead. There was significant variation in the starvation resistance and development rates of flies reared on the different diets. A tradeoff between these two traits was observed in flies reared on most of the diets, except for carrots, spaghetti squash, and limes.
At the time of transfer, I also stored some flies for microbiome analysis, to determine the conventional microbiota composition of flies reared on each diet. Currently, I am working to sequence the microbiomes of these flies to guide my creation of a gnotobiotic bacterial mixture to use in follow-up experiments that will compare the development rate and starvation resistance of axenic ('bacteria-free') and gnotobiotic flies on these same diets. These follow-up experiments will allow me to test how the diet affects the life history of the flies, both dependent upon and independent of the fly microbiota. Diet-dependent life history variation in axenic flies will reveal which diets sustain fly development and maturity independent of the microbiota. Life history values and microbiota composition in gnotobiotic flies will reveal interactions between the diet and the microbiota, including which combinations maximize particular life history traits. Together, these findings help establish how and why natural variation in the diets and microbiota composition of *D. melanogaster* can contribute to life history variation in locally adapted fly populations. More broadly, understanding these factors will help establish an explanatory framework for how host-microbe interactions influence animal evolution.

261B **Molecular and transcriptional characterization of a physical niche mediating symbiotic gut microbiome colonization in** ***Drosophila melanogaster*** *Haolong Zhu*[1,2], Allan Spradling[1,2,3], William Ludington[1,2] 1) Department of Embryology, Carnegie Institution for Science, Baltimore, MD; 2) Department of Biology, Johns Hopkins University, Baltimore, MD; 3) Howard Hughes Medical Institute, Chevy Chase, MD

A mechanistic understanding of gut microbiome colonization is fundamental to understanding the microbiome's functions and its physiological relevance. Specific associations with certain microbiome species in a host's gastrointestinal tract have been consistently observed among many animal species including fruit flies, mice, and humans. However, little

is known about how these specific microbiome species are chosen for stable colonization and how such specificity is determined.

We hypothesize that the host gut actively promotes stable microbiome colonization with appropriate species via the molecular production of the appropriate environment, termed the "commensal niche." Maintaining the commensal niche for certain microbiome species can be physiologically important for effective food digestion, efficient nutrition absorption, and enhanced immunity.

Previously, we isolated the fly gut microbiome strain, *L. plantarum* (*LpWF*), from a wild fruit fly. The strain preferentially colonizes the fly foregut region, in particular the proventriculus inner lumen, with high stability, suggesting a commensal niche for *LpWF* is produced by the fly. We took advantage of this strong colonization phenotype to ask how the commensal niche for *LpWF* is developed and maintained at the cellular and molecular levels. We applied bulk and single-cell RNAseq to profile the transcriptional changes in the spatially defined commensal niche over time to identify specific cellular and molecular components essential for appropriate microbiome colonization. We are evaluating the identified candidates through loss-of-function and gain-of-function experiments.

In summary, our study seeks to decipher the molecular basis of gut microbiome colonization and how it benefits the host. Such a mechanistic understanding is likely analogous to other host-microbiome interactions as a general paradigm and could enable the design of intervening strategies for a probiotic gut microbiome that benefits human health.

262C Microbiota effects on climbing abilities in *w*^{1118} flies *Tanner B. Call*[1], Paige E. Bonnette[2], John M. Chaston[3], Shaleen B. Korch[4], Gerald B. Call[4] 1) Arizona College of Osteopathic Medicine, Midwestern University, Glendale, AZ; 2) Biomedical Sciences Program, College of Graduate Studies, Midwestern University, Glendale, AZ; 3) Department of Plant and Wildlife Sciences, College of Life Sciences, Brigham Young University, Provo, UT; 4) Department of Pharmacology, College of Graduate Studies, Midwestern University, Glendale, AZ

It is known that the microbiota can affect motor function in *Drosophila melanogaster*. We undertook an experiment to test the effects of different bacterial mono- and combination-associations in *w*^{1118} in our novel climbing assay. This assay uses the TriKinetics Multibeam Monitor to record the location of 16 individual flies every second over a 20-minute period. Analysis of the data can reveal multiple climbing and motor parameters. Using the standard bleaching protocol, *w*^{1118} embryos were made axenic (germ-free) and were then mono-associated with *Acetobacter pomorum*, *Acetobacter tropicalis*, *Lactobacillus brevis*, or *Lactiplantibacillus plantarum*. Another group of *w*^{1118} flies were associated with a combination of all four bacteria (Combo). Interestingly, the Combo group had the most significant effects on climbing metrics compared to the axenic control group, including increases in average height climbed, number of climbs, and total height climbed, which were unchanged by any of the mono-associations. The amount of time spent by the flies in the lower tube portion was decreased in both the Combo and *A. tropicalis* groups. These two groups also had an increased amount of time spent in the upper tube portion, suggesting that *A. tropicalis* increases the ability and or motivation of the flies to climb to the top of the tube and stay there longer. Both of the *Acetobacter* mono-associations and the Combo group had increased total movements and passes through the middle of the tube, indicating that the *Acetobacter* mono-associations promote increased locomotion in general. Interestingly, the *L. brevis* and *L. plantarum* mono-associations had no effects on climbing. This detailed analysis of climbing behavior in this study reveals some interesting findings. First off, the combination of all four bacteria had the most effects on climbing, indicating that the mixed microbiota in conventional flies likely promotes substantial influences on climbing. This finding shows that the host climbing phenotype is determined by community interactions in the microbiota and not by one or more community members acting individually. Second, the *Acetobacter* species appeared to be the most significant contributors to the effects observed with the Combo group, while the *Lactobacillus* species had no effects on climbing.

263A Effects of host genetic feeding preferences in shaping microbiota composition in D. melanogaster *Caroline Massey*, John Chaston, Maggie Johnson Brigham Young University, Provo, Utah

The microorganisms within a host, commonly referred to as the microbiota, play an important role in the development of an organism and their life history traits, including fecundity and lifespan. In the model organism Drosophila melanogaster host genotype can significantly alter the microbiota composition, meaning that host genotype can determine at least in part which microorganisms are present and how abundant they are. What is not fully understood are the mechanisms by which host genotype selects the microbiota composition. In this work, I seek to better understand how the genetic feeding preferences of D. melanogaster help determine host microbiota composition. I have been using an assay where I will measure the variation in the microbiota composition of different fly populations when they are given a choice of different microbes in their diet; or when no choice is provided. This work will contribute to our understanding of how host genotype influences the microbiota variation observed between genetically distinct organisms.

264B The Influence of Lab Manipulated Fermented Fruit and Maternally Inherited Microbiota on Metabolic Phenotype *Oluwatobi Fijabi*[1], Graham Jones[1], Derek Maas[1], Andrei Bombin[2], Laura Reed[1] 1) University of Alabama; 2)

Vanderbilt University Medical Center, Nashville, TN

The infectious and pandemic nature of obesity is known to have a multifaceted root cause and must be addressed in a multivariate manner. Recent efforts have implicated the gut microbiome to be correlated with obesity-related phenotypes in *Drosophila*. Our previous research explored the influence of the natural microbiota of rotten peach fruit consumed by *Drosophila* in the wild in comparison to the conventional corn-based lab diet. In this present study, we are particularly interested in peach (P) and strawberry (S) diets because of the presence of health-enhancing phytochemicals, dietary fibers, and unsaturated fatty acids. Therefore, we investigated the influence of microbiota in shaping obesity-related phenotypes in *Drosophila* by testing the influence of genotype, maternal, and dietary microbiota in rotten P and S diets in shaping life history and metabolic traits. Organic P and S fruits were naturally fermented for six days and assessed for microbial and phytochemical composition through 16SrRNA sequencing and GC-LS/MS, respectively. We sterilized embryos with hypochlorite and ethanol to eliminate maternal bacteria on the chorion before larval emergence and treated the fermented P and S diets with autoclaving and antibiotics. Autoclaving is a thermal and pressure treatment that transforms and denatures nutrients but eliminates all microbes present. Conversely, the antibiotics eliminate 99% of microbes without heat application, keeping the nutritional integrity of the food. Hence, we adopted a novel strategy of rearing larvae of three DGRP lines on the naturally fermented P and S diets simulating the wild environment and on autoclaved and antibiotic P and S diets, giving us insight into the interactions between these treatments and our measured phenotypes. We anticipate that our results will reveal similar microbial compositions in P and S diets in beta diversity and major taxa. We also predict that larvae raised on P and S diets will have significantly higher survival, weight, triglyceride, and faster developmental time which are obesity-related phenotypes compared to the autoclaved and antibiotic P and S diets. In sum, we expect to see significant interactive effects between diet, parental microbiome and fly genotype in shaping metabolic phenotypes of larvae

265C The influence of environmental factors on the composition of fruit fly microbiota. *Reese Hunsaker*, John Chaston Brigham Young University, Provo, Utah

The microorganisms that are associated with a host are called the microbiota and can provide key benefits to their hosts. For example, the microbiota of the fruit fly *Drosophila melanogaster* strongly influences fly life history traits and evolution. Variation in the composition of fruit fly microbiota can influence or determine fly fecundity, life span, and starvation resistance. Importantly, we have observed variation in *D. melanogaster* microbiota composition in flies that naturally adopt different life history strategies, establishing a link between these fly characteristics. However, one key gap is that we do not know which processes determine variation in microbiota composition in wild fly populations. The goal of this research is to test if three possible environmental factors can influence the abundance of bacterial species in the *D. melanogaster* microbiota. To do this, I am rearing gnotobiotic flies, inoculated from birth with six bacterial species common to wild flies in conditions that vary by one of three major environmental parameters: temperature, humidity, and photoperiod. Then, I measure changes in whole body microbiota composition of adult flies. I have already performed several temperature replicates and these data showed a strong influence on fly microbiota composition. I still need to perform humidity and photo period replicates. Together, these approaches will show which environmental conditions have the greatest effect on fly microbiota composition.

266A Evaluating Approaches for Bacterial Mono-association in Parkinson's disease Model *Drosophila melanogaster* *Paige E Bonnette*[1], Gerald B Call[2] 1) Department of Biomedical Sciences, College of Graduate Studies, Midwestern University, Glendale, AZ; 2) Department of Pharmacology, College of Graduate Studies, Midwestern University, Glendale, AZ

Parkinson's disease (PD) is a neurodegenerative disease commonly associated with motor symptoms. Additionally, PD patients often suffer from non-motor symptoms, including gastrointestinal issues such as constipation and gut dysbiosis. These gut manifestations in a neurodegenerative disease support the hypothesis of a gut-brain-axis, a bi-directional communication pathway between the central nervous system and the gut. To investigate this relationship, our lab has been performing bacterial mono-associations in a PD model ($park^{25}$) *Drosophila melanogaster.* The $park^{25}$ flies are an excellent PD model as they possess many similar phenotypes to PD, such as dopaminergic neuron loss and impaired motor function. To perform a mono-association experiment, the flies must first be made germ-free, or axenic. The most frequently used method of rearing axenic *Drosophila* is by embryo dechorionation. This process generally consists of rinsing embryos with a 0.6% bleach solution followed by sterile water rinses and placement onto a sterile diet. Following this, our approach to collect homozygous $park^{25}$ axenic flies involved transferring sterile, homozygous pupae (identified by the absence of the Tubby phenotype) to new sterile diet tubes. To create gnotobiotic adult flies, we performed two bacterial inoculations: the first with the embryos and the second onto the sterile diet that the pupae were transferred on to. While this method did produce successfully mono-associated $park^{25}$ flies, the adult survival rate was low. Therefore, we wanted to see if there was a more efficient and effective method to perform mono-association experiments. We began by maintaining axenic fly stocks of both our control (w^{1118}) and $park^{25}$ flies. Axenic status of the stocks was checked periodically throughout the experiments. To perform a mono-association from the axenic stocks, adult axenic flies were allowed to deposit embryos for three days on new, sterile food. When the adults were removed, the embryos and larvae

present on the food were inoculated with *Lactiplantibacillus plantarum* or maintained axenic. Pupae were transferred from these tubes and placed into new sterile diet tubes without a second inoculation. Approximately 5-6 days post-eclosion, flies were homogenized, and bacterial colonies were cultured and counted to determine the average CFUs/fly. Despite there being one less inoculation event compared to the standard protocol, this method showed that the flies colonized at a similar level to previous experiments in our lab. Compared to our initial protocol, the axenic stock method with the single inoculation event colonized equally in w^{1118} flies (96.4%), or even better in $park^{25}$ flies (266%). Preliminary results suggest that this new method is similar to the traditional dechorionation method with regard to eclosion, adult survival, and motor function (climbing ability), which will be reported at the meeting.

267V Transcriptional Profiling of Immune Priming in *Drosophila melanogaster* *Kevin Cabrera*[1,2], Duncan Hoard[1], Zeba Wunderlich[1,2] 1) University of California, Irvine; 2) Boston University

Studying the way organisms fight off infections is a universally useful endeavor: anything that is alive has the possibility of getting sick. The historical paradigm in immunology focused on the binary distinction between the innate and adaptive immune systems. Increasingly, this binary convention has been challenged by the observations primed innate immune responses in organisms with and without an adaptive immune system. This so-termed "immune priming" helps organisms more effectively fight off a second infection after survival of an initial infection. Despite the wide prevalence of immune priming across many clades from plants to insects to mammals, much of the mechanistic work on this phenomenon has focused on cell-based mammalian models and lacked transcriptional characterization in insects. Here, we use *Drosophila melanogaster* to create a powerful*, in vivo* model for studying epigenetic control of immune priming. By infecting flies with an insect-derived strain of the Gram-positive bacterium *Enterococcus faecalis*, we modeled infection response and quantified enhanced survival in immune primed cohorts. We also tracked bacterial load over time and found preliminary evidence showing that the enhanced survival in primed cohorts does not correlate with differences in bacterial load. Using RNA-seq, we have tracked transcriptomic changes associated with immune priming in the primary immune organs of *D. melanogaster*, the fat body and hemocytes. Using differential expression analysis, we classified families of genes that remain activated throughout experiment, more efficiently re-activate upon re-infection, or are qualitatively unique to a primed immune response. Delineating the relative contributions of each of these mechanisms not only reveals the drivers of infection survival, but also suggests epigenetic mechanisms of gene regulation and tradeoffs between the immune response and other biological processes. By integrating gene expression with chromatin remodeling events and the effect of gene deletion on priming ability, we begin shortlisting regulatory elements that may be driving primed immune response. In this way, we unveil a concerted mechanism explaining immune priming in the fly.

268V Establishing the feasibility of *Drosophila melanogaster* as a model system for *Acinetobacter baumannii* infection Parvin Shahrestani, Maria Soledad Ramirez, *Melanie Garcia* California State University, Fullerton

Acinetobacter baumannii is an antibiotic-resistant bacterium of public-health concern. Current model systems used to study *A. baumannii* infection are limited. My objective is to establish the feasibility of the common genetic model organism, the fruit fly (*Drosophila melanogaster*), as an alternative for studying *A. baumannii* infection. Preliminary data revealed that *D. melanogaster* inoculated in the thorax with a pin-needle dipped in *A. baumanni* suspension had statistically lower survival than controls. Although, survival was still approximately 95%. Using a nanoinjector to apply higher infection doses resulted in further reductions in post-infection survival in a dose-specific manner. A strain of *A. baumannii*, AMA, thought to be more pathogenic in humans in comparison to the model strain A118 was seen to be more pathogenic against *D. melanogaster*. These results support the feasibility of using *D. melanogaster* to study this dangerous human pathogen. In upcoming work, we will distinguish between tolerance to and resistance against *A. baumannii* and test the impacts of antibiotics on post-infection host condition. Establishing *D. melanogaster* as a model to study *A. baumannii i*nfection will advance current understanding of antibiotic resistant mechanisms, immune defense evasion, as well as assist with drug development.

269V The role of host microbiota in aging of *Drosophila melanogaster* *Courtney Mueller*, Parvin Shahrestani California State University, Fullerton

Laboratory selection can cause large phenotypic differences in eukaryotic populations without the introduction of new mutations. A key potential contributor to an animal's evolution could be the host's microbiota, however this element has been largely ignored in previous laboratory studies. Indeed, many host traits that can evolve in response to laboratory selection, can also be influenced by the microbiota. Moreover, when populations undergo laboratory selection for divergence in health-related traits, they also become differentiated in their microbiota. What evolves first? Do host traits evolve before the host microbiota changes or does the microbiota change as a result of the evolution of the host phenotype? I hypothesize that if the host phenotype evolves before the microbiota, then populations that are selected for fast development will evolve before changes to the microbiota. Using laboratory selection, I will evolve fast-developing populations from slow-developing populations. Each generation, for at least 10 generations, I will monitor development time and host genetic control of the microbiota. I predict that when populations

of *Drosophila melanogaster* are selected for fast development, changes to development time evolve before changes to the microbiota. In other words, the microbial changes are a consequence of host evolution rather than a contributor to host evolution. Efforts to uncover the mechanisms of fast evolution are widely studied without the microbial component. Here is one of the first looks into how microbiota affect development time of *D. melanogaster*.

270V Metabolic regulation of blood progenitor homeostasis and heterogeneity by TCA cycle in development and immune response in Drosophila larvae ajay kumar 1) Institute For Stem Cell Science and Regenerative Medicine (inStem); 2) The University of Trans Disciplinary Health Sciences and Technology (TDU)

Immunity as well as metabolism are quite old and extensively focused and sort after fields, but their inter-dependency, as Immune- metabolism, is being advocated very recently. This crosstalk infers how under immune compromised state, metabolic shift occurs and metabolites (a-KG, Succinate, Fumarate, etc.) takes on the tasks of proliferation, differentiation and activation of the concerned immune progenitors/cells. Recent work from our lab also highlighted GABA (released from brain) can elicit distinct immune cell population, under wasp infestation, which is nowhere to be seen in homeostasis (Madhwal et. al., 2020). Through our work we would like to switch the conventional paradigm of looking at TCA cycle as just an "intermediary step in the glucose metabolism for energy production" to the "reservoir of cardinal immune metabolites". Metabolites are well known to get exchanged between the cellular compartments, which brands them competent for being signalling molecules. And what could be the better place for studying metabolites than TCA cycle, which is source as well as sink for all the three macromolecules of life. Conventionally TCA cycle, as the name suggest, always considered to be a cycle, but is it actually a cycle or an important junction of various cycles, so as to facilitate the exchange as well as maintenance of constant concentration of each metabolite. If the later is true, then each of the metabolites generated here can be an independent signalling molecules and directs the heterogeneity of different system. Here with our thorough analysis of TCA cycle, we would like to shed some light onto the crosstalk between the intermediary metabolites, development of blood progenitors and the heterogeneity produced within.

271V Amyloid Beta Peptide Plays an Immune Role in Alzheimer's Disease Pathogenesis *Nguyen Le*, Ashley Waring, Emma Hartness, Nathan Mortimer Illinois State University, Normal, IL

Alzheimer's Disease (AD) is a neurodegenerative disease characterized by severe memory decline and cognitive impairments. In AD patients' brains, aggregates of amyloid beta (Aβ) peptides, called amyloid plaques, are hypothesized to trigger innate immune responses that contribute to AD pathogenesis. Interestingly, recent studies have demonstrated that an increased level of Aβ protects the host against pathogen infections. Using Drosophila as a model organism, we find that the loss of the Drosophila APP homolog, APPL, leads to immune deficits against parasite infection, and that overexpression of Drosophila Aβ produces an inflammatory phenotype. These findings suggest that Aβ might be required for a successful immune response. We additionally aim to examine whether there is a correlation between Aβ aggregation and inflammatory responses and whether Aβ-mediated inflammation is associated with cognitive defects. We will produce flies that develop different levels of Aβ aggregation and examine the inflammation responses of these flies during a pathogen infection. In addition, a fly oviposition assay will be used to examine the cognitive functions of the Aβ-expressing flies. This study will help to shed light on how innate immunity and infection contribute to the development of AD.

272V Immune role of *Drosophila melanogaster* Kazal-type serine protease inhibitor CG14933 *Alexandra Hrdina*[1], Shu Kondo[2,3], Igor Iatsenko[1] 1) Max Planck Institute for Infection Biology, Berlin, Germany ; 2) Department of Biological Science and Technology, Faculty of Advanced Engineering, Tokyo University of Science, Tokyo, Japan; 3) Invertebrate Genetics Lab, National Insitute of Genetics, Mishima, Japan

Serine protease inhibitors (serpins) exhibit major regulatory functions in the proteolytic cascades of both arthropods and mammals. In *Drosophila melanogaster,* two of the major immune responses, the Toll pathway and the melanization cascade, are tightly regulated by serpins. Despite their known function in immunity, only a few of the 30 genes predicted to encode for serpins have been characterized, and knowledge about their exact mechanism in which they control the immune response to prevent excessive activation is still scarce. In this study, we investigate the immune role of the previously uncharacterized *Drosophila* putative serpin CG14933 containing a Kazal domain. By using $CG14933^{SK1}$ flies, a mutant devoid of any *CG14933* expression, as well as RNAi knockdown and deficiency models, we show that flies lacking the gene show an increased susceptibility to *Pseudomonas entomophila* infection but not to any other infections that we tested. This clearly indicates an involvement of *CG14933* in *Drosophila* immunity, although *CG14933* itself is not induced by infection. We demonstrate that CG14933 has no role in the regulation of major immune pathways and does not act as a neutralizer of bacterial proteases. Additionally, we found that in adult $CG14933^{SK1}$ flies, active phenoloxidase levels are significantly increased compared to wild type flies. We were able to rescue the sensitivity phenotype to *P. entomophila* infection by employing a double mutant devoid of both *CG14933* and *PPO1,* the prophenoloxidase activated in early stages of infection. Moreover, by using tissue-specific RNAi-mediated silencing of *CG14933* we reveal a significant role of the gene in the fat body of *Drosophila*. Altogether, we propose that CG14933 plays a role in negatively regulating the melanization response in *Drosophila.* Furthermore, we provide evidence of a possible link between excessive

melanization and increased susceptibility to *P. entomophila* infection.

273V Gut barrier defect and hyperactivation of innate immune response in a *Drosophila* model of NGLY1 deficiency *Ashutosh Pandey*[1], Seung Yeop Han[1], William F Mueller[2], Benjamin A Story[2], Antonio Galeone[3], Lars Steinmetz[2,4], Hamed Jafar-Nejad[1] 1) Department of Molecular & Human Genetics, Baylor College of Medicine, Houston, TX-77030, USA; 2) Genome Biology Unit, European Molecular Biology Laboratory (EMBL), Heidelberg, Germany; 3) Department of Bioscience, University of Milan, Milan, Italy ; 4) Department of Genetics, School of Medicine, Stanford University, Stanford, CA, USA

Mutations in human *N*-glycanase 1 (NGLY1) cause a congenital disorder of de-glycosylation (CDDG) known as NGLY1 deficiency. It is a multisystem disorder with symptoms including global developmental delay, lack of tears, movement disorders, and chronic constipation. However, the biological roles of NGLY1 and pathophysiology of NGLY1 deficiency are not well understood. The *Drosophila* homolog of human NGLY1 is encoded by *Pngl*. Loss of *Pngl* results in semi-lethality. Previous reports from our group have shown developmental and functional defects in *Pngl* mutant intestine due to impaired BMP signaling and reduced AMPKα level, respectively. However, the lethality in *Pngl* mutants is only partially explained by these affected pathways (BMP and AMPK). This suggests the potential contribution of other biological pathways to the lethality in *Pngl* mutants. To investigate this, we performed RNAseq on the midgut tissue of *Pngl* mutant larvae and observed mis-regulation of a number of gene categories. In agreement with previous studies, proteasomal genes constituted one of the top down-regulated gene categories. Notably, major up-regulated gene categories were related to immune response, leading to the hypothesis that hyperactivation of innate immune response might contribute to the lethality in *Pngl* mutants. Reducing the innate immune signaling by decreasing Toll and immune deficiency (IMD) pathways rescued the lethality of *Pngl* mutants by 20-23%. Decreasing the gene dosage of the forkhead box O family transcription factor Foxo (reported to induce immune genes expression in *Drosophila* midgut upon stress) rescued ~40% of lethality. We observed increased activation of Foxo in the *Pngl* mutant larval enterocytes. Fluorescently labeled dextran feeding assay showed gut barrier defects in *Pngl* mutant larvae. Pharmacological disruption of peritrophic matrix (a major component of the gut barrier in flies) by feeding polyoxin D to wild-type larvae showed mild lethality and increased expression of innate immune response genes. Taken together, our data present evidence for gut barrier defects and hyperactivation of innate immune response in a *Drosophila* model of NGLY1 deficiency.

274V The Impact of Increasing Concentrations of Ragweed Pollen on the Innate Immune System and Allergic Response of *Drosophila melanogaster* Shaila Sachdev Princeton High School, Princeton, NJ

The global warming crisis, caused by human activities, not only affects the environment but also human health. The increase in average annual temperature leads to longer growing seasons and higher pollen concentrations leading to an increase in the development of asthma and atopic allergies. Ragweed pollen happens to be the primary allergen trigger around the globe and affects more than 25 million Americans. *Drosophila melanogaster* exhibits a similar inflammatory response as humans making it a useful replicate asthma model. Although fruit flies only possess an innate immune system, they are outstanding invertebrate model organisms because they have comparatively simple physiology which allows for easy manipulation while possessing the major organs relevant to asthma. For instance, their trachea is made entirely of epithelial cells and directly exposed to high oxygen pressure and environmentally produced reactive oxygen species (ROS). It is believed that these reactive oxygen species may be generated by pollen which plays a potentially major role in influencing inflammatory responses in the airway epithelium. The purpose of this research is important to understand the extent of ragweed pollen's ability to induce an allergic reaction in a live biological species. To model this experiment, I will be varying the concentration of ragweed pollen that the fruit flies are exposed to in their food media during their third instar larvae stage. Healthy larvae are usually buried in their food, but upon oxygen deprivation -- due to increased pollen concentration -- they move to the surface, which suggests impairments of the tracheal system. This behavior will be quantified by measuring the percentage of larvae showing this behavior, which will provide information about the availability of oxygen to the epithelial tissue. If *Drosophila melanogaster* are exposed to ragweed pollen, then they should exhibit oxygen deprivation by remaining at the surface of the food media because ragweed pollen is a reactive oxygen species and presents major mediators of inflammatory responses in the airway epithelium.

275V In vivo demonstration of polymorphisms in antimicrobial peptides shaping host-pathogen interactions *Mark Hanson*, Lena Grollmus, Bruno Lemaitre EPFL

Antimicrobial peptides (AMPs) are key players in innate defence against infection. In *Drosophila*, a large array of immune peptides contribute to host defence downstream of the Toll and Imd NF-κB pathways. Our recent work using single and compound AMP mutations confirmed that AMPs can additively or synergistically contribute to combat pathogens in vivo. For instance, Drosocin, Attacin, and Diptericins collectively contribute to defence against *Providencia burhodogranariea*. However we also revealed a high degree of specificity wherein one AMP can play a major role in combatting a specific pathogen. We found a specific importance of Drosocin for defence against *Enterobacter cloacae*, and previous work has shown a primary role for Diptericins in defence against *Providencia rettgeri*. We also recently showed how AMPs shape the fly microbiome over aging, wherein AMP-deficient flies suffered increased microbiota load and community diversity

mimicking flies lacking Imd immune signalling. Finally, we recently described how furin cleavage enables a polypeptide AMP gene to produce multiple distinct peptides under the regulation of a single promoter.
Here we take advantage of newly-available mutations to dissect the specificity of these interactions in an isogenic genetic background. We confirm the Drosocin peptide specifically confers defence against *Enterobacter cloacae*, but also investigate a previously uncharacterized Drosocin peptide downstream of its furin cleavage site. This undescribed peptide specifically contributes to defence against *P. burhodogranariea*, while the classic Drosocin peptide does not in fact contribute to this defence. Moreover a Threonine/Alanine polymorphism in this uncharacterized Drosocin peptide mirrors the effect of gene deletion in defence against *P. burhodogranariea*. Thus the Drosocin gene confers defence against different pathogens using two distinct peptide products.
At the same time, we dissect the contribution of the two Diptericin genes in defence against *P. rettgeri* and other bacteria. We confirmed previous findings that Diptericin A is specifically important in defence against *P. rettgeri*. Surprisingly, we also uncovered a highly specific interaction between Diptericin B and systemic infection by a member of the gut microbiome. This finding of alternate specificites for the two Diptericin genes in defence against environmentally-relevant microbes paints a picture for how the immune system encodes innate defences. We show not only how AMPs and their alleles are critical to defence against specific natural enemies, but also provide a needed example for how specificity in defence can be derived from the evolution of AMP gene sequence and structure.

276V Does varying investment in egg production modify immune defense in mated female *Drosophila melanogaster*? *Kathleen Gordon*[1], Mariana Wolfner[2], Brian Lazzaro[1,3] 1) Department of Entomology, Cornell University, Ithaca, NY; 2) Department of Molecular Biology and Genetics, Cornell University, Ithaca, NY; 3) Cornell Institute of Host-Microbe Interactions and Disease, Cornell University, Ithaca, NY

In *D. melanogaster* and many other species, female reproductive investment comes at a cost to immunity and resistance to infection. In previous work, we have shown that *D. melanogaster* females become more susceptible to bacterial infection within hours of mating and for at least ten days. During mating, the male transfers seminal fluid proteins that change many aspects of female physiology and behavior, including inducing a rapid and sustained increase in egg production. One of these seminal proteins, Sex Peptide (SP), is involved in reducing female immune capacity. At least some of this effect may be due to stimulation of egg production by SP, as female flies without a germline, and who therefore produce no eggs, retain high immune capacity upon mating. Production of yolk protein to provision eggs is one of the major demands of reproduction, and we hypothesized that the level of investment in yolk protein production might directly or indirectly trade off with immune capacity. First, we ask whether high levels of production of yolk proteins (YPs) in the fat body, occasioned by increased egg production, interferes with that same tissue's ability to produce antimicrobial peptides (AMPs), the main effector molecules of the *Drosophila* immune system. In ongoing experiments, we are testing fertility and immune phenotypes of unmated or mated females with missense mutants in the three YP genes (Tanaka et al. 2021) and of null mutations in YP genes that we are generating with CRISPR-Cas9 genome editing. If removing YP production allows a higher immune response, we will test whether the SP-mediated increase in juvenile hormone (JH) levels in mated females is the basis for this tradeoff, as JH is known to stimulate YP production and decrease immune response. We will additionally test whether post-mating immune capacity is rescued by mutations that block early or late oogenesis. Together, these results can inform the mechanisms through which egg provisioning affects female immune response and generates a reproduction-immunity tradeoff.

277V Not quite FedEx: How are venom proteins packaged for delivery by the parasitoid wasp *Ganaspis hookeri*? *Nicholas Bretz*, Chris Lark, Nathan Mortimer 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 preliminary evidence suggests that venom proteins are packaged into venom-specific vesicles known as venosomes. These venosomes allow venom proteins to gain access to plasmatocytes likely via the interaction between virulence factors present on venosomes and host factors on the surface of plasmatocytes. Ultracentrifugation of *G. hookeri* venom separates venom proteins into unique fractions. Experiments utilizing nanoparticle tracking analysis and dynamic light scattering suggest that two of these fractions are composed of a heterogenous mix of vesicles, further supporting the idea that venoms can be stored as cargo within venosomes. With this, we are using fluorescence labeling of putative venosomes, mass spectrometry and SERCA activity assays to further characterize the venom transport mechanism. Vesicle packaging and specified targeting is a burgeoning field of research with potential applications in drug delivery for disease treatment.

278V Microbiome remodeling influences *Drosophila* immune response across generations *Krystal Maya-Maldonado*, Nichole A. Broderick Johns Hopkins University

In insects, including *Drosophila melanogaster*, the microbiome can exert intergenerational effects on host physiological processes through impacts on reproduction and development. In addition, specific microbiota members have been shown to influence mating, reproductive behavior, and development across generations.
Relatedly, previous studies in *Drosophila* and other insects have shown that immune status can be transmitted to progeny, a process referred to as transgenerational immunity. While several mechanisms of transgenerational immunity have been proposed, these have focused mainly on the transfer of an immunological experience to the offspring. Immune status is also known to be influenced by the microbiome, but the role of microbiota in transgenerational immunity has not been as well studied.
To address this knowledge gap, here we examined how interactions between a host, its' microbiome, and gut pathogen (s) modulate host immunity and microbiome composition across generations. First, we analyzed how the parental microbiome influences outcome in offspring immunity. We used the well-standardized protocols in the *Drosophila* model to get germ-free flies (axenic (AX) flies), and we compared them with conventional reared (CR) flies, which contain a native microbiota. Parents from each condition (CR or AX) were challenged orally with *Pseudomonas entomophila*, and the offspring were reared to the next generation. In the offspring, females were challenged with *P. entomophila* orally and we recorded survival, pathogen bacterial load, microbiome composition, and gene expression to analyze how the offspring respond to the challenge. This strategy was applied across two generations (offspring adults in F1, and F2). We found that *P. entomophila* persist in food where F1 develops, causing changes in microbiome composition of both the food and in the offspring. Interestingly, when we challenge F1 adults with *P. entomophila* we observed a better survival in the offspring from parents that were challenged, although pathogen bacterial load is the same between the offspring from parents' challenge and unchallenged. Our results suggest an immune tolerance effect is mediated by microbiome alterations across generations. Future studies will further characterize this mechanism of tolerance, as well as examine whether there have been genetic or transcriptional changes in the microbiome or pathogen.

279V Role of Juvenile hormone in mediating trade-offs between immunity and reproduction *Vanika Gupta*, Brian Lazzaro Cornell University

Immunity and reproduction are processes that reciprocally limit each other in a wide diversity of organisms. Mating stimulates the production of juvenile hormone (JH) by *Drosophila melanogaster* females, and JH is immunosuppressive. Thus, *D. melanogaster* females suffer reduced immunocompetence as a consequence of mating. JH additionally promotes fecundity and also plays a vital part in nutrient mobilisation in insects. The *D. melanogaster* genome encodes two JH receptors, *germ cell expressed* (*gce*) and *methoprene-tolerant* (*met*), which have different expression patterns and distinct functions. Previous reports indicate that *met* regulates fecundity while *gce* controls post-mating immunosuppression. Using tissue-specific knockdown of the two receptors *gce* and *met,* we further define the role of these receptors in determining the interaction between reproduction and immunity as mediated by the immunosuppressive properties of JH.

280C Comparative sex chromosome evolution in *Drosophila robusta* species group *Kamalakar chatla*, Doris Bachtrog University of California Berkeley

Neo-sex chromosomes frequently arise within the Drosophila genus through fusions between the ancestral X and Y chromosomes with autosomes. Typically, newly formed neo-Y chromosomes degenerate and become heterochromatinized, and the gene loss creates selective pressure for the homologue (neo-X) to acquire dosage compensation mechanisms to maintain proper gene dosage in males. Systematic, comparative studies are needed to study the dynamics of sex chromosome evolution, including heterochromatinization of neo-Y and dosage compensation on neo-X. In order to dissect the molecular mechanisms and evolutionary pressures driving the differentiation of these neo-sex chromosomes, we generated high-quality reference genomes, including Y chromosomes, using Nanopore single molecule sequencing combined with Hi-C scaffolding for several species with neo-sex chromosomes of varying age. We sequenced *D. nigromelanica*, *D. melanica*, *D. robusta* and *D. lacertosa* whose neo-sex chromosomes formed between 4.6 MY to 15 MY ago. The assembled Y chromosomes sizes varied from 10 Mb to 65 Mb which is inversely proportional to the age of neo-Y chromosomes. Homology to the neo-X and gene density decreased with Y age. *D. nigromelanica* harbors the youngest neo-Y chromosome maintaining 59% of genes, while the oldest neo-Y chromosome in *D. lacertosa* has less than 5% of genes remaining. All four species showed gene decay with accumulation of repetitive DNA on the neo-Y chromosome, and tandem amplification of some protein-coding genes. The neo-Y chromosome of *D. nigromelanica* is almost double the size of the ancestral autosome due to massive acquisition of repetitive DNA and tandem amplification of protein-coding genes. The three species carrying the older neo-sex chromosomes have fully dosage compensated neo-Xs that acquired male-specific lethal (MSL) complex binding sites. In contrast, *D. nigromelanica's* neo-X is not fully dosage compensated yet. Overall, our high-quality genome assemblies help to dissect the evolutionary trajectory of neo-sex chromosomes differentiation.

281A Effects of epigenetic silencing of transposable elements on local recombination rate *Yuheng Huang*, Grace Yuh Chwen Lee UC-Irvine

A wide range of taxa shows a negative association between transposable elements (TEs) density and meiotic recombination rates across genomes. Such association is commonly thought to be caused by reduced efficacy of selection against TEs in regions of low recombination or direct modification of recombination rates by TEs. Much of the research on TE evolution has focused on the selection explanation, leaving the latter possibility is largely unexplored. One possible mechanism by which TEs impact local recombination rates is through the modifications of chromatin environments. To reduce the selfish replication of TEs, hosts have evolved mechanisms to epigenetically silence them through the enrichment of heterochromatic marks at TEs. And it has been demonstrated that the enrichment of heterochromatic marks suppresses recombination initiation. Indeed, across the genomes, the heterochromatin environment is positively correlated with TE density and negatively correlated with recombination rates among different taxa. Therefore, we hypothesize that the TE-mediated heterochromatin enrichment can suppress the local recombination rate. To test the hypothesis, we aim to identify the associations between the distribution of recombination and epigenetically silenced TEs in three inbred strains with distinct TE insertion profiles. To measure recombination rate at a fine scale, we developed a novel approach that uses long-read sequencing to identify recombinant haplotypes in pooled individuals. We collected F1 offspring from two crosses and backcrossed them to the shared paternal line. For each cross, we collected more than 6000 F2 offspring and sequenced them with PacBio in a pool, aiming to identify crossover events using the long reads. This approach mitigates the need of sequencing individual flies in the traditional approach for constructing recombination maps. To benchmark this approach, we also sequenced 192 F2 flies individually with Illumina short-reads as well as in a single pool using PacBio long-reads to assess the sensitivity and specificity of our proposed new approach. We will then measure the enrichment of a repressive heterochromatic mark (H3K9me3) around the TEs and test their associations with recombination rates.

282B Evolution of Drosophila glue adhesiveness *Manon Monier*, Virginie Courtier Institut Jacques Monod, Université de Paris, CNRS, Paris, France

Bioadhesives display remarkable physico-chemical properties that allow living organisms to stick to a great variety of surfaces. Drosophila larvae produce a glue to attach themselves to a substrate for several days during metamorphosis. This glue is mainly composed of 7 Salivary gland secretion (Sgs) proteins and Eig71Ee protein. Alignments of *Sgs1, Sgs3, Sgs7, Sgs8* and *Sgs3bis* genomic regions across diverse Drosophila species reveal that *Sgs3* has a high rate of duplication and deletion. Contrary to most of Drosophila species that attach to wood, leaves, or dry fruit skin, *Drosophila suzukii* larvae attach to fresh fruit skin or into the soil. *Drosophila suzukii* is an invasive pest species and represents a threat for fruit crops worldwide. We found that *D. suzukii* and it's closely-related species *D. biarmipes* adhere poorly to glass compared to *D. melanogaster*. Our current analysis suggests that this weak adhesion is due to a lower amount of glue produced by salivary glands. Our work paves the way for a better understanding of the genetic basis of glue adhesion and for future industrial applications.
Keywords: bioadhesives, *Drosophila suzukii*, adhesion, pupa, genes synteny

283C Reconstructing the evolutionary history and neofunctionalization of the ZAD-Znf chromatin regulator *dwg* *Jack Jurmu*[1,2], Andrew Arsham[1,2] 1) Bemidji State University; 2) North Hennepin Community College

The ZAD-Znf family of genes is evolutionarily dynamic, expanding sporadically across insect lineages via duplication and retrotransposition. The family is characterized by the presence of a conserved N-terminal zinc-coordinating ZAD domain upstream of anywhere from one to over a dozen grouped C2H2 zinc finger domains, connected by a poorly defined "linker" domain. New or fast-evolving ZAD-Znf genes can become essential through neofunctionalization. For example, *nicknack* and *oddjob*, two genes within a cluster of five ZAD-Znf paralogs, are essential in *Drosophila melanogaster* and have recently gained a heterochromatin regulatory function. *Deformed wings* (*dwg, aka zeste-white5 or zw5)* and *CG2712* are neighboring and paralogous ZAD-Znf genes on the X chromosome of *D. melanogaster.* Protein sequence analysis suggests that both have acquired new functions within the last 5 million years. While *dwg* plays an essential role regulating insulators and enhancers in development, little is known about its non-essential near neighbor *CG2712*. In collaboration with the Genomics Education Partnership, we annotated the coding sequences of the two genes in *D. melanogaster* and other *Drosophilid* lineages diverging up to 40 million years ago. We compared domain and sequence architecture in order to reconstruct their evolutionary history and investigate their recent neofunctionalization.

284A Horizontal transfer of an apoptosis-inducing toxin gene in an agriculturally destructive fruit fly genus *Saron Akalu*, Kirsten Verster, Noah Whiteman University of California - Berkeley, Berkeley, California

Horizontal Gene Transfer (HGT) is the movement of genetic material between species, as opposed to via transmission from parent to offspring (i.e. vertical gene transfer). A growing body of evidence underscores the role of HGT in animal evolution. We previously discovered HGT of an apoptosis-inducing gene, *aip56*, into the nuclear genomes of the tephritid fruit fly genus *Bactrocera*, a genus that includes destructive fruit pests. In this study, we characterize the timing and evolution of *aip56* HGT in the *Bactrocera* lineage. We use PCR, Sanger sequencing, and analysis of publicly accessible genome assemblies to determine that *aip56* was transferred into the nuclear genome of a *Bactrocera* ancestor ca. 63

mya, and is highly expressed in the fat body. Additionally, we analyze the structure of AIP56 in *Bactrocera* using Phyre2 and find that the C-terminus has a high-confidence match to a rhamnose-binding domain, which suggests an immune function. Our results suggest that HGT of *aip56* served an adaptive function that may have facilitated the success of this agriculturally devastating clade.

285B The Conservation of The GlyP Gene Across highly divergent species of Drosophila *Bethany Lieser*, Reece Lawlor, Bao Khang, Alyssa Beise, Paula Croonquist Anoka-Ramsey Community College

The Insulin/Tor signaling pathway is responsible for the uptake of glucose into cells and its metabolism. It has also been linked to cell growth, fat and protein metabolism, and longevity. Its dysregulation in humans plays a major role in Type II Diabetes, Cardiovascular Disease, and cancer. GlyP, a member of the Insulin signaling pathway, encodes for the glycogen phosphorylase enzyme which is responsible for glycogen breakdown in the cell by releasing glucose into the bloodstream. Previous evidence has indicated that a gene's selective constraint is influenced by its protein connectivity and position in pathway, among other factors, so that genes with less molecular interactions and a closer location to the membrane evolve faster than those with more protein-protein interactions and located closer to the nucleus. We hypothesized that GlyP would exhibit high selective constraint in *D. busckii, D. hydei, D. kikkawai, and D. suzukii* when compared to *D. melanogaster*, the reference species, due to its high connectivity and downstream position in the insulin pathway. The GlyP gene was annotated in each species utilizing the Genomics Education Partnership (GEP) tool pipeline, namely, the UCSC Genome Browser, tBlastn, Blastp, the Gene Record Finder, and verified in the Gene Model Checker. Genes models were proposed based on synteny, RNAseq data and other lines of evidence. The protein alignment of GlyP in all species was examined. All species were within 97% similar to the reference species despite *D. busckii* being the furthest diverged species from *D. melanogaster*. This supports our hypothesis that highly connected genes, also known as hubs, are under high selective constraint even in species vastly separated by evolutionary time. The gene's downstream position may also provide evidence that GlyP is highly conserved and a cornerstone enzyme in the insulin pathway.

286C A genome wide model for estimating DNA transposable element excision rates in *Drosophila virilis* *Stefan Cerbin*[1], Danny Miller[2,3], Justin Blumenstiel[1] 1) Department of Ecology and Evolutionary Biology, University of Kansas, Lawrence, KS; 2) Department of Genome Sciences, University of Washington School of Medicine, Seattle, WA 98195, USA; 3) Department of Pediatrics, Division of Genetic Medicine, University of Washington and Seattle Children's Hospital, Seattle, WA

DNA transposons are sequences that are capable of moving in the genome resulting in profligate DNA damage and genome instability. Estimating transposon mobilization rates and the damage they cause is important for understanding genome evolution and genome stability. *Drosophila virilis* has several strains with varying copy number of DNA transposons and differences in germline piRNAs profiles. Transposons become activated when females lacking the requisite piRNAs to unique transposons inherited paternally, resulting in hybrid dysgenesis. With these strains we can study global transposon mobilization to determine the rates of excision and the overall change in copy number. In this study, we use pooled long-read DNA sequencing to identify elements mobilized in dysgenic crosses. These reads will be analyzed to estimate an excision rate using a likelihood model for estimating global excision rates for DNA transposons. This model will incorporate specific DNA transposon insertions, family, and piRNA profiles as parameters. We predict that differences in piRNA profiles, DNA transposon family identity, location, and internal deletion status will jointly determine the excision rate.

287A Testing the Effects of Fast-Evolving Heterochromatic Genes on Euchromatic Transposable Elements in Drosophila *Leila Lin*, Yuh Chwen G. Lee UC Irvine

Eukaryotic genomes consist of gene-rich, transcriptionally active euchromatin and gene-poor, silenced heterochromatin. The tightly packed heterochromatic regions make up at least 20% of the human genome and about one-third of the fruit fly (Drosophila) genome. Heterochromatic genes play a central role in maintaining the stability of the genome. Specifically, their protein products are involved in the epigenetic silencing and compartmentalization of repetitive sequences and transposable elements (TE) that are enriched in heterochromatin. Accordingly, we hypothesize that heterochromatic genes must be fast evolving in order to keep up with the rapidly-changing and oftentimes deleterious repetitive sequences and transposable elements in heterochromatin. Supporting this hypothesis, we have identified fast-evolving heterochromatic genes across sixteen species of Drosophila flies. We achieved this through performing copy number variation analysis, a phylogenetic analysis by maximum likelihood (PAML) to test evolution across long evolutionary time, and a McDonald-Kreitman test (MK) to test evolution across short evolutionary time. To test whether the fast evolution of these heterochromatic genes may also influence euchromatic TEs that are epigenetically silenced with enrichment of heterochromatic marks, we crossed flies with a mutation for the genes of interest to a reporter strain with red fluorescent marker next to with various families of TEs. Changes in the intensity of the fluorescence would provide information about the extent of epigenetic silencing of TEs in the euchromatic genome. Our study will connect how the fast-evolving gene-poor heterochromatin may shape the evolution of gene-rich euchromatin.

288B Predicting Gene Essentiality in Non-Model Drosophila Species to Understand Phenotypic Evolution of New Genes *Dylan Sosa*, Manyuan Long Department of Ecology & Evolution, The University of Chicago, Chicago, USA

Essential genes, which lead to lethality if functionless, are conventionally presumed to be conserved and ancient, whereas young genes are considered dispensable and nongermane to organismal survival. New genes, which are evolutionarily young, have been found to have essential functions in diverse processes such as centromere targeting, gametogenesis, human brain development, reproduction, and protein diversification. As new genes retain characteristics of the evolutionary forces that engendered them, their structures, and their functions; they provide a unique opportunity to gain insight into the evolution of essential functions.

Empirical investigations of essential genes on a whole-genome scale via functional genomic experiments such as RNAi are laborious, expensive, typically available only for model species, and are hardly suitable for complex organisms such as humans or mice. This has resulted in a deficit of functional and phenotypic data for understudied non-model species, limiting our understanding of the evolution of essential gene functions. The intractability of creating knock-out lines for non-model species, or infeasibility in the case of complex organisms, has necessitated the ability to accurately and precisely predict essential genes *in silico* as candidates for functional interrogation and evolutionary analysis.

In this work, I will develop machine learning methods to utilize features extracted and patterns learned from *D. melanogaster* 3rd generation sequencing-based assemblies and phenomic data to predict and identify essential new genes in non-model *Drosophila* species as targets for experimental and evolutionary analyses. Specifically, I will develop deep learning and ensemble learning algorithms to predict essential genes in non-model species followed by gene age dating to identify new genes and their putative origination mechanisms. I will then conduct CRISPR/Cas9-based knock-outs of candidate essential new genes to validate their essentiality by measuring lethality and fertility effects in the knock-out lines I create. This work will yield insight into the evolution and development of gene essentiality in both model and non-model species, as well as provide open-source computational software for predicting essential new genes in *Drosophila* species with the potential for application in other non-model organisms.

289C Extensive genome-wide homozygosity tracts reveal micro-environment population structure in *Drosophila* populations. *Peter Andolfatto*[1], Clair Han[2,3], Patrick Reilly[2,4], Andrew Taverner[2], Ana Pinharanda[1], Sheel Chandra[1], Kevin Deitz[5], Daniel Matute[6] 1) Columbia University; 2) Princeton University ; 3) HHMI Janelia ; 4) Yale University; 5) American Museum of Natural History; 6) University of North Carolina Chapel Hill

Drosophila population samples are usually established as descendent populations of a single wild-caught female ("isofemale lines"). As a result, whole genome sequencing (WGS) data has generally been collected either from isogenized versions of isofemale lines or from pools of wild-caught individuals. Both methods represent a departure from the approach used for most other organisms, which is to sequence outbred diploid individuals. In a population genomic survey of *Drosophila santomea*, an island endemic sister-species of *D. yakuba*, we re-sequenced the genomes of 34 wild-caught individuals widely distributed across the island of Saõ Tomé. Plots of population-level genome-wide diversity reveal nothing particularly remarkable. Despite this, closer inspection reveals unusually long runs of homozygosity (ROH) within individuals. Remarkably, a substantial proportion of these individuals (>75%) harbor more ROH than expected for offspring of matings at the level of 1st cousins or closer. The level of diversity between individuals greatly exceeds that of within individuals (F_{IT} = 0.194), strongly suggesting non-random mating. This is particularly surprising given the small geographic scale over which these samples were collected and indicates the existence of a large number of extremely small micro-environments on the island. The general lack of WGS of outbred diploid individuals in *Drosophila* prevents the easy identification of such ROH patterns. Among the rare exceptions is *D. sechellia*, for which wild collected individuals exhibit non-random mating patterns similar to that of *D. santomea*. Simulations reveal that the most likely demographic model for both species is one of many extremely small populations (resembling isofemale lines) with low but non-zero migration between them. Our findings have important implications for the fine-scale structure of *Drosophila* populations and downstream population genomic inference of demographic and selection parameters.

290A SR drive and the evolutionary history of the Y chromosome *in Drosophila simulans* *Cecile Courret*[1,2], David Ogereau[2], Amanda Larracuente[1], Catherine Montchamp-Moreau[2] 1) University of Rochester; 2) Laboratoire Evolution Génome Comportement et Ecologie, CNRS UMR9191, Gif sur Yvette and Université Paris-Saclay

Meiotic drive is an infringement of the law of equal allele segregation into the gametes. In heterozygote individuals, the causal genetic elements prevent the production of gametes that do not contain it. Thus, they can spread through populations even if they are deleterious for the carriers. Because they induce sex-ratio bias, sex-linked drivers expressed in the heterogametic sex are an important source of genetic conflict, characterized by the evolution of suppressors which tends to restore a balanced sex ratio.
In *Drosophila simulans*, X-linked meiotic drivers disturb the segregation of the Y chromosome during male meiosis. The progeny of carrier male is mainly composed of females. The drive is caused by two X-linked elements, acting together. The first one lies within a segmental duplication, the second has been identified as the HP1D2 gene, a young

and fast-evolving member of the Heterochromatin Protein 1 gene family. HP1D2 accumulates on the heterochromatic Y chromosome in spermatogonia, strongly suggesting that it controls the segregation of sister chromatids through heterochromatin modifications.
In natural populations where the drivers have spread, they are neutralized by resistant Y chromosomes. We followed the dynamics of the resistant Y chromosomes in natural populations in relation to the dynamics of the driver. We observed the replacement of sensitive by resistant Ys within a handful of years in populations invaded by the drivers. To go further in the study of Y chromosome variation in this species we sequenced 21 iso-Y lines, their Y chromosomes came from different locations around the world and have different phenotypes. We confirm the very low nucleotide diversity among Y chromosomes in this species, which could be considered as a signature of recurrent genetic conflicts. While we identified a haplotype with fixed differences between sensitive and resistant Ys. The sequence similarity between the sensitive Y chromosomes suggests that they have a recent common ancestor. We also confirm the ancestry of the resistant lineage by examining Y-linked sequences in the sister species of *D. simulans, D. sechellia* and *D. mauritiana*. All together the molecular polymorphism allows us to retrace the demographic and evolutionary history of the Y chromosome related to the one of the species. We show that intragenomic conflicts can drive astonishingly rapid evolution of Y chromosomes.

291B Natural Selection Shapes Variation in Genome-wide Recombination Rate in Drosophila pseudoobscura *Kieran Samuk*[1,2], Brenda Manzano-Winkler[1], Kathryn R Ritz[1], Mohamed AF Noor[1] 1) Duke University; 2) University of California, Riverside

While recombination is widely recognized to be a key modulator of numerous evolutionary phenomena, we have a poor understanding of how recombination rate itself varies and evolves within a species. Here, we performed a comprehensive study of recombination rate (rate of meiotic crossing over) in two natural populations of *Drosophila pseudoobscura* from Utah and Arizona, USA. We used an amplicon sequencing approach to obtain high-quality genotypes in approximately 8,000 individual backcrossed offspring (17 mapping populations with roughly 530 individuals each), for which we then quantified crossovers. Interestingly, variation in recombination rate within and between populations largely manifested as differences in genome-wide recombination rate rather than remodeling of the local recombination landscape. Comparing populations, we discovered individuals from the Utah population displayed on average 8% higher crossover rates than the Arizona population, a statistically significant difference. Using a Q_{ST}-F_{ST} analysis, we found that this difference in crossover rate was dramatically higher than expected under neutrality, indicating that this difference may have been driven by natural selection. Finally, using a combination of short- and long-read whole-genome sequencing, we found no significant association between crossover rate and structural variation at the 200–400 kb scale. Our results demonstrate that (1) there is abundant variation in genome-wide crossover rate in natural populations, (2) at the 200–400 kb scale, recombination rate appears to vary largely genome-wide, rather than in specific intervals, and (3) interpopulation differences in recombination rate may be the result of local adaptation.

293A *Acetobacter* to *Lactobacillus* Ratios within *Drosophila Melanogaster* Microbiota, Diet and Environment Across a Latitudinal Gradient *Aubrey Johansen*, Amanda Morrison, Emma Davis Brigham Young University, Provo, UT

The community of microorganisms associated with an organism ('microbiota') can be tightly linked to the evolution and life history variation of that organism in the wild. Therefore, patterns in the composition of these microbial communities are likely to be linked to the adaptive traits and evolution of their hosts. Our lab previously identified a pattern in the abundance of the two dominant bacterial groups in wild *Drosophila* from two locations in the United States: acetic acid bacteria (AAB) are more abundant at low latitudes and lactic acid bacteria (LAB) are more abundant at high latitudes. While this variation in microbiota composition can be attributed at least in part to host genotype, the likely role of environmental sources of microbes in determining the AAB:LAB ratio of wild flies have not been clearly defined. In this work we define how variation in environmental reservoirs of microorganisms are linked with variation in microbiota composition of the flies from two locations in the United States. We sampled flies, their diets, and soil from thirteen orchards located in the eastern United States and northern Utah, USA. We are currently working to sequence the microbiota of these samples and compare the LAB:AAB ratios between flies and their environmental substrates to determine if there is congruence in overall microbiota composition or abundance of specific microbial groups. Our early analyses on a subset of samples suggests the abundance of LAB in flies and their diets are coupled, although the initiating cause of this coupling has not yet been identified. Our continued work with these and additional samples will test if this pattern is reproducible in multiple locations and if other microorganisms also display covariation between flies and their diets. Together, these approaches will help define the role of environmental sources of microbes in determining microbial compositions in wild Drosophila populations.

294B Chromosomal Rearrangements in two populations of *Drosophila yakuba* *Timothy Ranallo-Benavidez*, Rebekah Rogers UNC Charlotte

Understanding how genetic mutations underlie population differentiation is an important question for evolutionary biology. Chromosomal rearrangements can produce new genetic sequences that lead to phenotypic differences

between closely related species. We have identified chromosomal rearrangements in two recently diverged populations of *Drosophila yakuba* from mainland Africa and the island of Mayotte. To understand the effect that a more contiguous reference sequence has on identifying chromosomal rearrangements, we also use a recent long-read *Drosophila yakuba* assembly. With this new reference we are able to identify many more mutations, many of which occur in centromeric regions.

295C Karyotype evolution - Insights from a *D. melanogaster* strain with unusual sex chromosome karyotypes *Duojia Li*[1], Dhyey Gandhi[1,2,3], Yukiko Yamashita[1,2,4] 1) Whitehead Institute for Biomedical Research; 2) Department of Biology, Massachusetts Institute of Technology; 3) Department of Chemistry, Massachusetts Institute of Technology; 4) Howard Hughes Medical Institute, Massachusetts Institute of Technology

Drosophila melanogaster utilizes the X/Y sex determination system where the X to autosome ratio determines sex. Their sex chromosomes, X and Y, each harbors a ribosomal DNA (rDNA) locus consisting of hundreds of tandemly repeated rRNA genes whose sufficient copy number and transcription is essential for cellular functions. Females carrying Y chromosomes are rare occurrences which usually results from low rates of nondisjunction events. Here we discovered a strain of *D. mel*anogaster from geographically isolated Seychelles archipelago whose X chromosomes barely contains rDNA, necessitating all females to carry at least one Y chromosomes. We found that this strain has two kinds of Y chromosomes, an intact one (Y) and a truncated one (Y^S) that lacks the majority of the long arm, the latter of which cannot support male fertility. Investigating why such a non-functional Y^S chromosome may be maintained in this strain, we found that Y chromosome makes female sterile when present in multiple copies. Therefore, Y^S serves as the essential source of rDNA for female. Our work on the Seychelles strain may hint at how karyotype may evolve, eventually leading to the diversification of species.

296A Tandem duplications as targets of selection in local adaptation *Taylor Conway*[1], Rebekah Rogers[2] 1) University of North Carolina at Charlotte; 2) University of North Carolina at Charlotte

Tandem duplications are a source of genetic novelty that create new gene sequences, alter gene expression patterns, and modify existing genes. We have used computational and molecular methods to describe evolutionary impacts from recently diverged populations in Drosophila. We locate and compare tandem duplications in *Drosophila yakuba* populations from mainland Africa compared with Island populations on Sao Tome and the eastern island Mayotte. These two independent cases of island invasion clarify how tandem duplications respond during habitat shifts. We have identified 1,426 mutations in *D. yakuba* capturing 1,022 genes. New gene structures include whole gene duplication and chimeric constructs. We are exploring the role these play in population differentiation in island environments.

297B Chromosomal rearrangements as a source of local adaptation in island Drosophila *Brandon Turner*[1], Theresa Miorin[2], Nick Stewart[3], Cathy Moore[1], Robert Reid[1], Rebekah Rogers[1] 1) University of North Carolina at Charlotte; 2) University of Georgia; 3) Fort Hayes State University

Chromosomal rearrangements act as a source of genetic novelty by shuffling DNA throughout the genome. These mutations can produce chimeric genes, induce de novo gene formation, or alter gene expression changes for existing genes. Here, we explore how these mutations may serve as agents of evolutionary change as populations adapt to new environments during habitat shifts. We identify 16,480 rearrangements in mainland D. yakuba and two locally adapted populations of D. santomea and D. yakuba on Sao Tome. Three loci that are associated with signals of strong differentiation in D. santomea lie adjacent to UV resistance or DNA repair genes, suggesting that these rearrangements confer selective advantages in high altitude environments with greater UV stressors. Some 55% of these mutations are facilitated by TE insertions, and 28% are TE facilitated ectopic recombination. In D. santomea 468 mutations are associated with strong signals of differentiation from the mainland while in island D. yakuba we identify 383 candidates of local adaptation. A total of 49.4% of mutations associated with signals of local adaptation also show signi cant changes in transcript levels, suggesting that the adaptive value of rearrangements is related to effects on gene expression. Together, this survey of structural variation identifies key modes of evolutionary innovation that would be missed in SNP-based screens. This work offers a portrait of how these mutations appear and help organisms to survive during habitat shifts, furthering our understanding of evolutionary processes.

298C Genetic variation in recalcitrant repetitive genomic regions in *Drosophila melanogaster* *Harsh G. Shukla*, Mahul Chakraborty, J.J. Emerson University of California, Irvine

About one third of the *Drosophila melanogaster* genome is heterochromatic and consists of repetitive sequences

like satellites, transposable elements (TE), ribosomal DNA, and occasional single-copy sequence. Despite its role in chromosome segregation, nuclear organization, and gene expression, much of the highly repetitive heterochromatin has been recalcitrant to assembly. This limitation has impeded delineation of genetic variation, evolution, and function of this crucial genomic region. To resolve the sequence of the repetitive genomic regions and map the genetic variation within them, we *de novo* assembled the genomes of two isogenic strains, the reference strain ISO1 and A4, using Pacific Biosciences highly accurate (HiFi) long reads and compared their assemblies. The euchromatin arms are gapless in our assemblies and provide a complete map of genetic variation in them. We incorporated ~8 Mb of new heterochromatin sequences into the chromosome arm scaffolds, including ~3.5 Mb of X pericentric heterochromatin containing rDNA. We also assembled ~15 Mb of Y Chromosome from the two strains, unveiling the first detailed map of genetic variation for this highly repetitive chromosome. We show that despite being prone to structural mutations, the repetitive regions of the *D. melanogaster* genome exhibit contrasting patterns of copy number variation across different gene arrays. For example, the size of the Histone cluster is similar (~560 kbp) between A4 and ISO1, whereas the X-linked *Stellate* (*Ste*) gene cluster shows striking variation between the two strains. The Histone cluster in both strains consists of ~110 copies, whereas A4 and ISO1 carry 192 and 11 tandem copies of *Ste* in the X euchromatin, respectively. The varying degrees of structural variation in these two gene clusters are likely because the Histone copy numbers are evolving under stabilizing or purifying selection, whereas the *Ste* copy numbers are shaped by an evolutionary arms race between X-linked *Ste* and their Y-linked suppressors *Su(Ste)*. Furthermore, complete resolution of tandem arrays like the Histone cluster at nucleotide level offer an avenue for determining the relative roles of birth-and-death vs concerted processes in the evolution of such clusters. Our results not only show a detailed map of molecular genetic variation within the hitherto unassembled repetitive genomic regions, but also lay the foundation for comparative and functional genomics of complete *D. melanogaster* genomes.

299A A tandem duplication in *Drosophila melanogaster* shows enhanced expression beyond the gene copy number *David Loehlin*, Caleigh Paster, Jeremiah Kim Williams College

Tandem duplicated genes are common features of genomes, but the phenotypic consequences of their origins are not well understood. It is not known whether a simple doubling of gene expression should be expected, or else some other expression outcome. We describe an experimental framework using engineered deletions to assess any contribution of locally-acting *cis*- and globally-acting *trans*-regulatory factors to expression interactions of particular tandem duplicated genes. *Acsx1L* (*CG6300*) and *Acsx1R* (*CG11659*) are tandem duplicates of a putative acyl-CoA synthetase gene found in *D. melanogaster*. Experimental deletions of the duplicated segments were used to investigate whether the presence of one tandem duplicated block influences the expression of its neighbor. *Acsx1L,* the gene in the left block, shows much higher expression than either its duplicate *Acsx1R* or the single *Acsx1* in *D. simulans*. *Acsx1L* expression decreases drastically upon deleting the right-hand duplicated block. Crosses among wildtype and deletion strains show that high tandem expression is primarily due to *cis*-acting interactions between the duplicated blocks. Sequence and phylogenetic analysis suggest that the duplication rose to fixation in *D. melanogaster* and has been subject to extensive gene conversion. Some strains actually carry three tandem copies, yet strains with three *Acsx1* copies do not have higher expression levels than strains with two. Surveys of tandem duplicate expression have typically not found the expected twofold increase in expression. This study suggests that *cis*-regulatory interactions between duplicated blocks could be responsible for this trend.

300B Seasonal plasticity and adaptive fluctuations of gene expressions of *D. melanogaster* *Yang Yu*, Alan Bergland University of Virginia

Two major mechanisms for populations of short-lived organisms to respond to temporal environmental heterogeneity, such as seasonality, are adaptive tracking and plasticity. Theory predicts that transition from one mechanism to another will have detrimental effects on the populations, thus the genetic architecture between adaptive tracking and plasticity should have limited overlap. Seasonal adaptive tracking can be observed from allele frequency change at individual loci and at genome-wide levels. Although thousands of SNPs have been shown to shift in frequency repeatedly across seasons in *Drosophila melanogaster* populations, we still have limited understanding of the relative strength of evolutionary mechanisms that underlie seasonal adaptation. In this study, we test the hypothesis that there is distinct genetic architecture between adaptive tracking and plasticity by using genome-wide plastic gene expression profiles across the season. We first identify the genes that plastically change in expression levels across the season to gain insight into the functional response to seasonal environmental changes. Second, we will combine publicly available eQTL profiles, genome-wide allele frequency data from multiple seasonal populations, with our seasonal gene expression data to test whether the eQTLs associated with plastic genes are de-enriched for seasonal SNPs. We created and reared a genetically controlled F1 fly population from 24h embryos to adulthood in an experimental orchard across 10 seasonal time points (May to Oct in 2019) to examine the differentially expressed (DE) genes across the season. We extracted whole-tissue RNA from 3-5-day-old adult female flies and prepared pooled libraries using bulk RNA barcoding method (BRBSeq). Our next step is to identify the plastic genes and test our hypothesis that the eQTLs associated with these genes are de-enriched for seasonal SNPs. We hope to provide insight into the general understanding of seasonal

adaptation from an expression perspective, and how seasonal environmental heterogeneity maintains functional genetic variation at eQTLs across time.

301C Shavenbaby as a model to link phenotypic and gene regulatory changes across *Drosophila* evolution *Tatiana Gaitan*[1], Kaelan Brennan[1], Julia Zeitlinger[1,2] 1) Stowers Institute for Medical Research, Kansas City, MO; 2) The University of Kansas Medical Center, Kansas City, KS

Phenotypic variability resulting from changes in gene regulation is often mediated by changes at cis-regulatory DNA sequences, i.e., enhancers. However, how changes in DNA sequence at enhancers generate different phenotypic outputs across evolution is not well understood. Understanding how transcription factors (TFs) read out cis-regulatory information at enhancers has been challenging, in part due to technical limitations associated with mapping TF binding at their motifs with sufficiently high-resolution to study crucial facets of transcriptional regulation such as, motif arrangement (syntax) and cooperativity between TFs. In the *Drosophila* embryo, axis patterning signaling defines the expression pattern of Shavenbaby (Svb), a key regulator of epidermal cell shape, which then induces the expression of cellular effectors that specify actin-rich projections called trichomes. How Svb directs the transcriptional activation of its targets presents an excellent opportunity for evolutionary analysis of motif syntax because of the epidermal phenotypes that are strikingly different between species. Thus, to interrogate the syntax of Svb targets, we are collecting genome-wide binding information in late-stage *D. melanogaster* embryos and applying our high-resolution ChIP technique, ChIP-nexus, to map TF binding at base-resolution. With these data, we observe high-resolution footprints of Svb at its own motif within known epidermal enhancers. To obtain predictive rules for Svb binding that we can apply in an evolutionary context, we are combining Svb ChIP-nexus data with BPNet, a deep learning model that uses DNA sequences to predict ChIP-nexus binding information. BPNet successfully learns Svb ChIP-nexus data and identifies motifs predictive for Svb binding, which map to known Svb enhancers, and uncharacterized regions that represent new candidate enhancers. Next, we are identifying suitable *Drosophila*species for evolutionary comparisons by performing cuticle preparations, Western blots, and immunostaining. With the strongest candidates, we aim to compare changes in motif syntax, and cooperativity with candidate TFs at target enhancers. Svb ChIP-nexus data together with BPNet provide an unprecedented opportunity to analyze neutral and functional changes in TF binding, and will further our understanding of how gene regulatory logic modulates phenotypic output across evolution.

302A Identification of Three Novel Paralogs of *CG3795* *Jaquelyn Hester*[1], Amanda Moy[2], Jayda Cavanaugh[3], Kaitlyn Schoonover[4], Kelly Alvarado[5], Stanley Guan[6], Evan Merkhofer[3], Gerard McNeil[6], Howard Granok[5], Martin Burg[2], Michael Foulk[4], Christopher Ellison[1], Wilson Leung[5], Cindy Arrigo[7] 1) Rutgers University - New Brunswick, New Brunswick, NJ; 2) Grand Valley State University, Allendale Charter Township, MI; 3) Mount Saint Mary College, Newburgh, NY; 4) Mercyhurst University, Erie, PA; 5) Washington University in St. Louis, St. Louis County, MO; 6) CUNY York College, Jamaica, NY; 7) New Jersey City University, Jersey City, NJ

The *CG3795* gene is located on the Muller A Element (X chromosome) in *Drosophila melanogaster*. The FlyBase Gene Summary indicates that this gene is involved in the breakdown of proteins (proteolysis) and exhibits serine-type endopeptidase activity. As part of an investigation into the expansion of the *Drosophila ananassae* Muller F Element (~19.1 Mb compared to ~1.3 Mb in *D. melanogaster*), we identified four features within the *D. ananassae* Oct. 2018 (AGI/DanaRS2) assembly that show significant sequence similarity to the *D. melanogaster* CG3795-PA protein. Two of the features were located on scaffold QMES02000001 (tentatively assigned to the Muller E Element based on synteny analysis), and two of the features were located on scaffold QMES02000178 (tentatively assigned to the Muller D Element). Upon further analysis, one of the features on scaffold QMES02000178 was assigned as the putative ortholog based on the following criteria: highest protein alignment coverage (i.e., subject coverage), low E-value, and high percent identity. The other features were determined to be novel paralogs of *CG3795* since the RNA-Seq data shows that these regions are being actively transcribed in *D. ananassae*. In addition, the BLASTX alignments of the *D. ananassae* genomic region surrounding each feature against the *D. melanogaster* CG3795-PA protein did not show any in-frame stop codons or frame shifts, thereby supporting the hypothesis that these features are protein-coding genes, not pseudogenes. The RNA-Seq data in *D. melanogaster* suggests that *CG3795* is a male-specific gene, with it's highest expression levels in the testis (modENCODE Tissue Specific RNA-Seq data and Developmental RNA-Seq data). In contrast, the modENCODE RNA-Seq data indicates that *CG3795* is not expressed in embryos or in adult females. This *CG3795* male-specific RNA-Seq data expression pattern can also be observed in *D. ananassae*. Comparative annotation of species closely-related to *D. ananassae* shows that the *CG3795* ortholog and the three *CG3795* paralogs are also present in *D. bipectinata*. Given the number of novel paralogs of *CG3795* found in both *D. ananassae* and *D. bipectinata*, we expect neofunctionalization of these male-specific genes in these two species. Future investigations will try to identify the *CG3795* orthologs and paralogs in other *Drosophila* species in order to better understand the evolution of this male-specific gene.

303B The evolution of morphology at a single-cell resolution *Ella Preger-Ben Noon*, Yifat Yanku, Anna Urum, Stav Naky Technion - Israel Institute of Technology, Haifa, Israel

The evolution of animal body form results from genome divergence. How genome divergence is translated into the

morphogenetic events that generate new phenotypes is one of the most challenging questions in modern evolutionary biology. We address this question by studying the evolution of male genitalia in *Drosophilids.* Many anatomical features of the genitalia of *D. melanogaster* and its close relatives exhibit striking differences in shape and size, providing an excellent system to study the genetic, molecular and developmental basis of phenotypic evolution. Genetic mapping studies revealed that these differences are affected by multiple loci, but the evolved genes and how they coordinately function to generate diverse structures remains unknown.
The emergence of single-cell genomics provides an unprecedented opportunity to resolve these problems. Here, we use single-cell RNA-seq to generate gene expression atlases of genital discs of *D. melanogaster* and its sibling species *D. simulans.* This approach allows us to unbiasedly identify genes that are differentially expressed in evolved genital substructures between the two species. By combining unsupervised cell-clustering with published gene expression pattern data we obtained transcriptomes for each of the anatomical substructures of the genital disc and identified all the evolved genes within these substructures. Functional analyses using the powerful genetic toolkit of *Drosophila* will determine the relevance of these genes to male genitalia development and evolution.

304C New Transcript Formation in Hybrid Drosophila *Rebekah Rogers*[1], Nicholas Stewart[2], Cathy Moore[1] 1) University of North Carolina, Charlotte, NC; 2) Fort Hays State University, Hays, KS

The origin of new genes is among the most fundamental processes underlying genetic innovation. The substrate of new genetic material available defines the outcomes of evolutionary processes in nature. Historically, the field of genetic novelty has commonly invoked new mutations at the DNA level to explain the ways that new genes might originate. In this work, we explore a fundamentally different source of epistatic interactions that can create new gene sequences in hybrids. We observe “bursts” of new gene creation in F1 hybrids of D. yakuba and D. santomea, a species complex known to hybridize in nature. The number of new genes is higher in the gonads than soma. We observe asymmetry in new gene creation based on the direction of the cross. Greater numbers of new transcripts form in the testes of F1 male offspring in D. santomea female x D. yakuba male crosses and greater numbers of new transcripts appear in ovaries of F1 female offspring of D. yakuba female x D. santomea male. These loci represent wholly new transcripts expressed in hybrids, but not in either parental reference strain of the cross. We further observe allelic activation, where transcripts silenced in one lineage are activated by the transcriptional machinery of the other genome. These results point to a fundamentally new model of new gene creation that does not rely on the formation of new mutations in the DNA. These results suggest that bursts of genetic novelty can appear in response to hybridization or introgression in a single generation. Ultimately these processes are expected to contribute to the substrate of genetic novelty available in nature, with broad impacts on our understanding new gene formation and on hybrid phenotypes in nature.

305A More than molting: Ecdysone signaling in adult *Drosophila* *Zachary Drum*, Joseph Coolon Wesleyan University

Ecdysone signaling is critical for successful life-cycle transitions in developing holometabolous insects, but the role of ecdysone signaling in adult insects is understudied. Previous work in adult *Drosophila melanogaster* has found roles for ecdysone signaling in many different processes like stress responses, courtship behavior, reproduction, and lifespan, but the ecological relevance of ecdysone in adults is not well understood. Our work examining the transcriptional response of *Drosophila sechellia* to the toxic volatiles found in its host plant *Morinda citrifolia* has predicted both the ecdysone receptor (*EcR*) and the ecdysone induced transcriptional repressor *blimp-1* to be involved in the transcriptional response to these compounds. Altering the expression of these genes in adult *D. melanogaster* alters survival from octanoic acid (OA) toxicity. When *D. melanogaster* and *D. simulans* flies are fed food containing the active form of ecdysone (20E), their survival significantly increases and when *D. sechellia* flies are fed 20E, their survival on OA significantly decreases. The expression of genes involved in ecdysone response and biosynthesis are significantly different in these different fruit fly species, so examining basal ecdysone titers, 20E titers in response to OA exposure, and the genome wide transcriptional response to 20E by RNA-sequencing will help us understand if the ecdysone signaling differs in these species. Understanding how ecdysone signaling relates to OA resistance in *D. sechellia* may help elucidate how *D. sechellia* has adapted to specialize on the fruit of *M. citrifolia* and provide an ecologically relevant role of ecdysone in adult *Drosophila.*

306B Comparative Analysis of Node Degree on Gene Evolution in the Insulin Signaling Pathway *Abigail Myers*[1], Alyssa Koehler[2], Annie Backlund[3], Chinmay Rele[4], Laura Reed[5] 1) The University of Alabama; 2) The University of Alabama; 3) The University of Alabama; 4) The University of Alabama; 5) The University of Alabama

A biological pathway is a network of nodes (genes) interacting with each other and other regulating molecules in the cell which determines gene expression and therefore overall gene function of the cell.The insulin signaling pathway is extensively studied and includes a group of genes that regulate glucose metabolism. This, in addition to the pathway’s high conservation across species, makes it a beneficial model to study the function and evolution of biological pathways. In a network, node degree refers to the number of connections a gene has to other genes in the pathway. Previous studies using computational methods found genes with high node degree to be less evolutionarily constrained than genes with low node degree in this pathway. These results contradict the accepted hypothesis that genes with high node

degree are under more selective constraint because connected genes have to adapt to any mutations that occur at the node. In this project we further investigate the impact of node degree on gene evolution using manually curated gene models across the Drosophila genus. We are looking at the ratio of nonsynonymous to synonymous mutations (dN/dS) of three genes with varying node degree. We chose to focus on GlyS, raptor, and Dsor1; which are genes with low, intermediate, and high node degree, respectively. A comparative analysis of the nonsynonymous changes in these genes across Drosophila species related to their node degree will provide a small-scale analysis of the impact of node degree on gene evolution using manually curated gene models. This analysis will grow as more genes in the insulin signaling pathway are annotated and analyzed in the future.

307C *De novo* suppression of a male-harming mitochondrial mutation in *Drosophila melanogaster* via laboratory passaging *Sarah A. Tomlin*[1,2], Vada Becker[4], David M. Brinkley[3], Ching-Ho Chang[1,2], Harmit S. Malik[1,2] 1) Howard Hughes Medical Institute, HHMI, Chevy Chase, MD; 2) Fred Hutchinson Cancer Research Center, Basic Sciences, Seattle, WA; 3) University of Washington, Molecular and Cellular Biology, MCB, Seattle WA; 4) University of Washington, Molecular Medicine and Mechanisms of Disease, M3D Seattle, WA

Mitochondria are specialized double-membrane organelles of bacterial origin present in most eukaryotic cells. Mitochondria contain circular DNA (mtDNA) almost exclusively uniparentally (maternal) inherited through females in most plants and animals. Such uniparental inheritance has been hypothesized to result in the accumulation of 'male-harming' mutations that are neutral or beneficial to females due to lack of selection (the "Mother's Curse" hypothesis). Previously, we found one such exclusively male-harming mtDNA (G177S) mutation in the cytochrome oxidase II gene (COII) of *Drosophila melanogaster.* G177S led to significant impairment of male fertility at 25 and 29 degrees but no impairment in female function. Over five years, the G177S mtDNA mutation was passaged without selection in the w1118 nuclear background. We found that male fertility had recovered substantially but not to wildtype levels in this line, suggesting the possibility that a de novo nuclear suppressor of male infertility arose and swept to fixation in the laboratory. Intriguingly, restoration of male fertility appeared together with lower female fertility in flies carrying the G11S mtDNA mutation. To recreate the original male infertility phenotype, we take advantage of genetic backcrosses to a w1118 line not subject to selection because it carries wildtype mtDNA instead of G1777S mtDNA. We aim to identify the genetic basis of this de novo suppression by whole genome sequencing.

308A Maternal mRNAs underlie higher heat tolerance in tropical vs. temperate *Drosophila melanogaster* embryos *Emily Mikucki*, Thomas O'Leary, Brent Lockwood University of Vermont, Burlington, VT

The earliest stages of embryogenesis are particularly sensitive to environmental change, relative to other life stages. This phenomenon has been attributed to the transcriptional silence of early embryos, yet transcriptional responses to environmental change have not been fully characterized in the developing embryo. Previously, we demonstrated that tropical *Drosophila melanogaster* embryos have greater tolerance to acute heat stress than temperate North American embryos, suggesting that there is adaptive genetic variation in embryonic heat tolerance. In order to elucidate the molecular physiological basis of genetic differences in embryonic heat tolerance, we sequenced mRNA from early *D. melanogaster* embryos from tropical isofemale lines that were collected from 5 populations around the globe (Mexico, St. Kitts, Ghana, India, and Guam) and temperate North American lines collected from Vermont, USA. Early embryos (0-1 h old) were exposed to heat stress (32°C, 34°C, or 36°C) or control conditions (25°C) for 45 minutes prior to RNA sequencing. Out of ~29,868 sequenced transcripts, 828 (2.8%) differentiated the transcriptomes of tropical vs. temperate embryos, and all of these transcripts significantly correlated with heat tolerance among the lines. Functional enrichment analysis indicated that tropical embryos had higher abundances of maternally loaded transcripts that encode proteins involved in the oxidative stress response. We also found that tropical and temperate embryos exhibited similar changes in the abundance of 4,534 gene transcripts in response to temperature. 671 of these transcripts were induced by heat shock, indicating that embryos were transcriptionally active in the face of heat stress. Overall, our data indicate that tolerance to environmental perturbation during embryogenesis involves the oxidative stress response, corroborating recent studies on the important role of redox balance in fly development. Further, we demonstrate that transcriptional activity in the early zygote is context dependent.

309B Evolutionarily young, gene-silencing piRNA: innovation in gene regulation or control of selfish genetic elements? *Peiwei Chen*, Alexei Aravin California Institute of Technology

Every genome is colonized by transposons, a class of selfish genetic elements that can mobilize and replicate themselves within the host genome, causing DNA damage and genome instability. To cope with this, animals employ a small RNA-guided genome defense mechanism to silence transposons. Particularly, the piRNA pathway is responsible for the repression of transposons in animal germline. Though piRNAs have been reported to regulate targets other than transposons, the range of these non-transposon piRNA targets in different species remains poorly explored. Previously, we profiled the piRNA repertoire in the male germline of *Drosophila melanogaster* and found abundant piRNAs from a locus on the Y chromosome that target and silence a protein-coding gene on the X chromosome, *pirate*. Pirate is an evolutionary young gene that encodes a putative deSUMOylase. Intriguingly, we found that in

another *Drosophila* species, *D. mauritiana*, *pirate* is potentially repressed by another small RNA pathway, endogenous siRNA pathway, suggesting that two distinct small RNA-based silencing strategies were independently invented in recent evolution to regulate *pirate*. I will discuss our ongoing efforts in exploring whether *pirate* acts as a selfish genetic element and how different classes of small RNAs evolve to suppress *pirate* in the male germline.

310C Multi-trait genetic characterization of resistance to heavy metal stress *Elizabeth Everman*, Stuart Macdonald University of Kansas

Genetic characterization of stress response often relies on the assessment of a single trait; however, single trait assessments do not fully encompass an individual's response to stressors. We took a multi-trait approach to characterize the genetic factors that contribute to variation in resistance to copper toxicity by examining adult, developmental, and behavioral responses to copper stress. Copper is one of several common heavy metal pollutants that are leached into the environment through mining and agriculture practices. Copper is an especially interesting metal because it is required for normal physiological function and development at low concentrations, but at toxic levels copper exposure can lead to organ damage and failure as well as impairment of neurological function. Furthermore, our previous work has demonstrated that genes and pathways involved in detoxification of non-biologically necessary heavy metals (lead and cadmium) also contribute to variation in the response to copper toxicity. Using the approximately 1500 genetically stable strains that make up the *Drosophila* Synthetic Population Resource (DSPR), we previously identified 13 regions of the genome that contribute to adult physiological resistance to copper using quantitative trait locus (QTL) mapping. We are currently measuring developmental viability under copper stress in the DSPR, and preliminary QTL analysis indicates that the genetic control of adult and developmental copper resistance may be partially life stage specific despite the lack of a significant phenotypic correlation between the traits. Thus far, only one QTL is shared between the adult and developmental response to copper stress. The shared QTL highlights *mekk1* as a potential candidate gene, and estimated DSPR founder haplotype effects on developmental and adult responses to copper at this QTL suggests that allelic variation for *mekk1* in the DSPR may have similar effects on copper resistance in both life stages. In addition to multiple physiological traits, we measured variation in copper detection ability in 200 DSPR strains and found that the ability to detect copper at low concentrations in food is genetically variable. We also found that copper-tainted food consumption is negatively correlated with adult physiological resistance to copper stress, underscoring the importance of combining physiological and behavioral assessments of stress response. By measuring multiple traits related to copper stress in the same large panel of genetically stable strains, we will ultimately be able to reveal the genomic independence and interactions underlying multiple traits that shape the organismal response to chemical stress.

311A Discovering Zinc Resistance Loci via Extreme QTL Mapping *Katherine Hanson*[1], Anthony D. Long[2], Stuart J. Macdonald[1,3] 1) Department of Molecular Biosciences, University of Kansas, Lawrence, KS; 2) Department of Ecology and Evolutionary Biology, University of California at Irvine, Irvine, CA; 3) Center for Computational Biology, University of Kansas, Lawrence, KS

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. Since zinc is involved in many cellular functions, zinc toxicity can have widespread effects, leading to necrosis, inhibition of mitochondria, and impacting the homeostasis of other heavy metals. Zinc toxicity has been primarily studied via functional genetics, employing single-gene mutations and expression knockdowns in a small number of inbred lines. Because the response to zinc toxicity is a complex, polygenic, trait, 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 has orthologs of critical genes involved in zinc homeostasis, such as *MTF-1* (a transcription factor involved in metal response), zinc transporter (ZnT) proteins and has proved to be a successful model to understand other heavy metal response traits. To identify zinc resistance loci we employed extreme QTL, or XQTL, mapping, a powerful technique that identifies QTL via extreme phenotypic selection of a population. We established a large, outbred population by mixing hundreds of DSPR RILs (*Drosophila* Synthetic Population Resource Recombinant Inbred Lines), a set of advanced intercross RILs derived from 8 inbred founder lines. We raised animals from this population on media supplemented with toxic levels of zinc, sequencing the pool of surviving, zinc resistant females and a matching control population, replicating the experiment 12 times. We estimate that >4000 animals were tested per replicate, and in each case we selected the top ~7% of animals. At each position in the genome we estimated the founder composition from each pooled sample, identifying QTL as significant frequency shifts between control and selected populations. We succeeded in identifying seven QTL, including one that overlaps with a QTL previously identified for copper developmental viability. Our QTL encompass physical intervals between 320 kb and 880 kb and collectively contain 451 protein coding genes. We implicate *MTF-1*, *Mekk1*, a gene involved in cadmium toxicity, present within the shared copper/zinc QTL, and 5 genes found to be associated with zinc resistance in a *Drosophila* cell line study. For a majority of our QTL only 1 or 2 founder alleles show a substantial frequency change between the control and selected populations, implying that resistant and susceptible alleles are often rare. We identified a series of candidate genes using existing functional data, including

previous metal toxicity studies, and gene ontology, and will further test these via gut-specific RNAi knockdowns.

313C Correlating Regulatory Region and Genetic Evolution *Chinmay P. Rele*, Laura K. Reed The University of Alabama

Understanding change at an evolutionary scale is primarily done through accession protein and gene evolution; however, understanding how the regulation of those loci occurs would paint a clearer picture of how the proteome and genome interacts with the environment. The regulatory regions of genes are highly variable and important, for the timing of gene expression. Understanding the dynamics between the regulatory regions of an organism with its relevant effects in the genome, transcriptome, and proteome, can inform us of how the evolution of one plays a role on the other. We are studying the evolution of the regulatory regions of *Insulin-like peptides* (*Ilps*) and how their evolution correlates with other characteristics of the genes. *Ilp* is a good gene family to examine due to high duplication rates, and different mutation rates of those duplicates that occur within the *Drosophila* phylogeny. In this study, we use the hand-curated gene models of genes within the Insulin Signaling Pathway of 28 *Drosophila* species generated by The Genomics Education Partnership, including the *Ilp* paralogs. We are using these annotations to anchor the genomic region, extract genomic regions upstream of the start codon, and run Multiple Sequence Alignments. We intend to correlate features within these islands of conservation with genomic features to be able to find an association between regulatory regions and the genes they regulate. The patterns identified within these islands of conservation upstream of genes are likely cis-regulatory elements, or regions where regulatory elements exist that affect the regulation of the gene. Patterns such as indels (insertions/deletions), sequence motifs, as well as overall conservation found in these islands upstream of the gene can be correlated with the metrics of the genes directly downstream, such as ratio of nonsynonymous to synonymous mutations dN/dS, intron size, indel abundance, isoform number, and expression patterns (among other genetic characteristics). We expect to see positive correlation between the evolution of this upstream regulatory region and the evolution of features of the cis-regulated genes. Understanding the evolutionary patterns of these upstream regions is paramount in understanding how regulation of genes occurs.

314A What shall we do with the melanogaster species group? *Artyom Kopp*[1], Bernard Kim[3], Amir Yassin[2] 1) University California, Davis; 2) CNRS Laboratoire Evolution, Génomes, Comportement et Ecologie; 3) Stanford University

The genus *Drosophila* contains many hundreds of species that can be reared in the laboratory. This feature, along with the prominence of *D. melanogaster* as an experimental model system, has contributed to the widespread use of *Drosophila* as a model for molecular evolution, evo-devo, and comparative genomics. Ever since the 1930s, it has been recognized that classifying *Drosophila* species in a scheme reflecting their relatedness as well as their degree of divergence was fundamental for comparative studies. Therefore, the taxonomic rank of species groups was introduced and gained wide usage in *Drosophila* research. Of the nearly 60 species groups, the *melanogaster* group was initially created for 7-14 species since much of the diversity of Australasian and Afrotropical regions was unknown at that time. Over time, this group came to encompass ~200 species, far exceeding the typical size of other groups, and was subdivided into 10 subgroups. Recent phylogenetic studies revealed that those subgroups intermingle with several other species groups; thus, maintaining a monophyletic *melanogaster* group would require expanding its size and diversity even further. Some authors suggested upgrading the earliest branching *ananassae* (27 spp.) and *montium* (94 spp.) subgroups to species-group level, redefining the boundaries of the *melanogaster* group to a more reasonably sized clade. This solution simplifies the discussion of evolution within each lineage, as was done for example by subdividing the newly created *montium* species group into 7 new subgroups and 13 species complexes. On the other hand, this approach breaks with a huge body of comparative literature that includes *ananassae* and *montium* species as members of the *melanogaster* group. The aim of this poster is to discuss the taxonomic complexity of the *melanogaster* species group and to present through a balanced debate the benefits and disadvantages of different solutions. We also show that the *ananassae* subgroup/group is not monophyletic as currently defined, requiring additional taxonomic revisions.

315B Modelling Satellite DNA organization *Sherif Negm*, Amanda Larracuente Department of 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 studying estimating 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 capture the variation in repetitive satDNA arrays independent of copy number. We use Bayesian inference and regression approaches to infer recombination rate and gene conversion from simulated data and empirical data from a natural population of Drosophila melanogaster. We show that our approach could be useful for understanding how mutation, recombination and drift shape satDNA arrays under neutral evolution and selection.

316C Testing long-term evolutionary change and stasis in the pioneer factor Grainyhead *Henry Ertl*, Patricia Wittkopp University of Michigan, Ann Arbor, MI

A comprehensive understanding of morphological evolution must entail studying both evolutionary change *and* stasis in the developmental mechanisms that produce organismal form. Grainyhead (Grh) is a transcription factor critical for epithelial cell formation in the epidermis of flies, mice, and nematodes, suggesting the developmental role of Grh is conserved over 700 million years. To determine whether the underlying molecular functionality of Grh is also conserved, we constructed transgenic flies and tested whether the nematode Grh ortholog could rescue the weak epidermis phenotype of flies lacking functional Grh. We then collected gene expression, chromatin accessibility, and Grh binding data to provide a mechanistic explanation for any divergence between these distantly related Grh orthologs.

317A Rapid diversification shapes the evolution and function of sperm nuclear basic protein genes in *Drosophila* species *Ching-Ho Chang*[1], Isabel Mejia Natividad[1], Harmit Malik[1,2] 1) Division of Basic Sciences, Fred Hutchinson cancer research center, Seattle, Washington, United States of America; 2) Howard Hughes Medical Institute, Fred Hutchinson Cancer Research Center, Seattle, Washington, United States of America

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 sperm nuclear basic proteins (SNBPs). Although histones are ancient and highly conserved, SNBP repertoires have independent evolutionary origins, differ dramatically across animal lineages, and evolve rapidly in mammalian lineages. Here, we leveraged the sequencing of many *Drosophila* species' genomes to perform detailed phylogenomic and genetic analyses of SNBP genes. SNBP genes independently arose in *Drosophila* species via duplications of HMG (high mobility group) DNA-binding proteins. We found that 11 of 13 SNBP genes have higher protein evolution rates (dN/dS) than 95% of *D. melanogaster* genes. McDonald–Kreitman tests revealed that five SNBP genes have evolved under positive selection in the *D. melanogaster* lineage. Surprisingly, we found that the evolutionary conservation of SNBPs is not correlated with functional importance. Several ancestral, strictly retained SNBP genes are dispensable for male fertility in *D. melanogaster,* whereas two recently evolved SNBPs are essential for fertility. Moreover, SNBP genes necessary for fertility in *D. melanogaster* have been lost in other *Drosophila* species. To study the evolution of SNBPs, we ectopically express orthologs of one essential SNBP in *D. melanogaster* and found they express at different stages. Our result suggests that this SNBP might acquire function in mature sperm post-individualization and become essential. Moreover, we found extensive duplication of protamine genes across *Drosophila* species, with recurrent expansions of these duplications (>70%) on sex chromosomes. Our population genetic and phylogenetic analyses highlight that one X-linked SNBP amplification in *D. mauritiana* is under recurrent positive selection. However, the same amplification is independently lost and does not show the signature of positive selection in two sister species of *D. mauritiana*. These results indicate that sex chromosome-linked SNBP duplicates may be involved in either inducing or suppressing X-versus-Y meiotic drive. Our analyses suggest that the rapid evolution of SNBPs might be a universal phenomenon in animals and partly driven by genetic conflicts during spermatogenesis.

318B Molecular mechanisms underlying alternating cell polarity establishment in *Scaptodrosophila* follicle cells Miriam Osterfield UT Southwestern

Eggshell shape differs substantially among different species of drosophilids, with particularly obvious variation in the number of dorsal appendages. In *Drosophila melanogaster* for example, two patches of follicle cells are specified to an appendage fate, resulting in two dorsal appendages. In contrast, the follicular epithelium of *Scaptodrosophila lebanonenesis* (or *S. pattersoni)* contains only one patch of appendage-fated cells, but this patch gives rise to 4-8 separate dorsal appendages. Previous work has shown that these appendages are formed by the elongation of an alternating set of cell-cell edges within the linear floor cell domain. In other words, there is an alternating left/right pattern, so if a given floor cell lengthens on its left edge, its neighboring floor cells lengthen on their right edges. This study reveals a major molecular mechanism underlying this alternating cell polarity. A series of candidate approaches led to the identification of several molecules that localize specifically to the elongating subset of floor-floor edges; these molecules include Bazooka/Par-3, atypical protein kinase C (aPKC), F-actin, and phosphatidylinositol-4-phosphate (PI4P). In vitro treatment of *Scaptodrosophila* egg chambers with a panel of phosphatidylinositol kinase or phosphatase inhibitors revealed that PIK93 potently abolishes alternating localization of both PI4P and F-actin and also abolishes dorsal appendage formation. Based on the known selectivity of PIK93, this strongly suggests a role for a type-III PI4 Kinase, namely PI4KIIIbeta/Fwd or PI4KIIIalpha, in this process. Additionally, in vitro treatment of *Scaptodrosophila* egg

chambers with Latrunculin abolishes the alternating localization of both F-actin and PI4P. The apparent requirement of F-actin and PI4P for each other›s localization suggests that a positive feedback loop involving these components is important for selecting the subset of floor-floor edges that will elongate. Current work is focused on developing genetic tools in S*captodrosophila* to further examine this unexpected example of planar polarity.

319C Genomic analyses of new genes and their phenotypic effects reveal rapid evolution of essential functions in *Drosophila* development shengqian xia University of Chicago

It is a conventionally held dogma that the genetic basis underlying development is conserved in a long evolutionary time scale. Ample experiments based on mutational, biochemical, functional, and complementary knockdown/knockout approaches have revealed the unexpectedly important role of recently evolved new genes in the development of *Drosophila*. The recent progress in the genome-wide experimental testing of gene effects and improvements in the computational identification of new genes (< 40 million years ago, Mya) open the door to investigate the evolution of gene essentiality with a phylogenetically high resolution. These advancements also raised interesting issues in techniques and concepts related to phenotypic effect analyses of genes, particularly of those that recently originated. Here we reported our analyses of these issues, including reproducibility and efficiency of knockdown experiment and difference between RNAi libraries in the knockdown efficiency and testing of phenotypic effects. We further analyzed a large data from knockdowns of 11,354 genes (~75% of the *Drosophila melanogaster* total genes), including 702 new genes (~66% of the species total new genes that aged < 40 Mya), revealing a similarly high proportion (~32.2%) of essential genes that originated in various *Sophophora* subgenus lineages and distant ancestors beyond the *Drosophila* genus. The transcriptional compensation effect from CRISPR knockout were detected for highly similar duplicate copies. Knockout of a few young genes detected analogous essentiality in various functions in development. Taken together, our experimental and computational analyses provide valuable data for detection of phenotypic effects of genes in general and further strong evidence for the concept that new genes in *Drosophila* quickly evolved essential functions in viability during development.

320A Resolving the evolution and diversification of a *Hox*-regulated pigmentation trait *Ivan D. Mendez Gonzalez*[1], Mark Rebeiz[1], Thomas M. Williams[2] 1) University of Pittsburgh, Pittsburgh, PA; 2) University of Dayton, Dayton, OH

A fundamental question in evolutionary biology is how the diversity of forms and colors that we observe in the animal kingdom originated. *Hox* genes are highly conserved genes often implicated in phenotypic evolution, and yet the molecular mechanisms of *Hox* gene evolution have been difficult to pinpoint.
In *Drosophila melanogaster*, the *Hox* gene *Abd-B* regulates the production of melanin covering the A5 and A6 segments of the male's abdomen. This trait evolved from a non-melanic ancestor and in the *melanogaster* species group, within which it was extensively diversified. We tested whether temporal changes in *Abd-B* expression have played a role during the origination and diversification of abdominal melanic pigmentation. We identified two *cis*-regulatory elements (CREs) in *D. melanogaster* that are necessary for *Abd-B* expression in the A5 abdominal segment during late pupal development. Deletion of either CRE disrupts the formation of melanic pigmentation, suggesting that they have partially redundant activities. To test for intraspecific differences in the activity of these CREs, we replaced the *melanogaster* allele with the orthologous region from species with different melanic pigmentation. Our preliminary data suggests that the function of one of these CREs is highly conserved. Our current model is that partially redundant CREs form a relay to maintain *Abd-B* expression over time, and that evolutionary changes in this process that construct and deconstruct these CREs may be involved in the evolution of abdominal melanic pigmentation. Furthermore, the identification of these CREs offers the opportunity to manipulate *Abd-B* expression across different species to causally test how animal morphology evolves in response to changes in the expression of *Hox*-genes.
We propose that regulatory evolution of genes with multiple developmental functions, like *Abd-B,* should favor temporal rather than spatial changes, potentially limiting negative pleiotropic effects.

321B Germ granule analysis reveals conserved and diverse features among *Drosophila* species Dominique Doyle, Bianca Ulrich, Bianca Ortega, *Matthew Niepielko* Kean University

The co-packaging of different mRNA types into macromolecular structures called ribonucleoproteins (RNPs) is a conserved strategy for the regulation of mRNA metabolism. In many animals, the formation of complex RNPs called germ granules is essential for the post-transcriptional regulation of mRNAs that are required for germline development, maintenance, and function. In *Drosophila*, germ granules are assembled at the posterior of the egg and are inherited by the primordial germ cells during embryogenesis. In *D. melanogaster*, mRNAs accumulate in germ granules by forming homotypic clusters, which are distinct aggregates that contain multiple copies of a specific mRNA type. Homotypic clusters in *D. melanogaster* are generated through a stochastic seeding and self-recruitment process, relying on *cis*-regulatory sequences found in the 3'UTR of germ granule mRNAs called "clustering elements." We hypothesize that clustering elements may be susceptible to evolutionary changes, creating diversity in the abundance of mRNAs found in germ granules from different *Drosophila* species. To test our hypothesis, we first investigated the homotypic clustering of two germ granule mRNAs, *nanos* (*nos)* and *polar granule component* (*pgc*) in three *Drosophila* species. By

combining single molecule *in situ* hybridization (smFISH), super-resolution microscopy, and quantitative image analysis, we found that seeding and self-recruitment is a conserved process that generates homotypic clusters to enrich germ granules with mRNAs. Interestingly, we found that the mRNA content of *nos* and *pgc* homotypic clusters, as measured by absolute transcript number, were strikingly diverse among *Drosophila* species. Specifically, a 50% difference in the number of transcripts in *nos* and/or *pgc* homotypic clusters was discovered between *D. melanogaster*, *D. virilis*, and *D. pseudoobscura*. By employing computational modeling, we recreated the diversity in germ granule mRNA content from all three species. Our simulations suggest that a combination of factors, including differences in mRNA clustering efficacy, generates germ granule diversity. Currently, we are investigating if variability found in clustering elements underlies *Drosophila* germ granule diversity.

322C Reorganizations in the apical extracellular matrix underlie morphological diversification in *Drosophila* genital structures *Ben Vincent*, Lance Davidson, Mark Rebeiz University of Pittsburgh

Identifying the genetic changes that cause morphological differences between species is a major goal of evolutionary and developmental biology. While many groups have found success by investigating differences in pigmentation or the regressive loss of entire structures, we know less about the genes and pathways involved in the diversification of three-dimensional body parts. The posterior lobe in the *Drosophila melanogaster* clade is an ideal system to investigate morphological evolution – this genital structure exhibits staggering diversity among the *Drosophila melanogaster* subgroup, including the sister species *Drosophila mauritiana* and *Drosophila simulans,* and we can track its development by dissecting and staining pupal terminalia. Previous work has shown that the posterior lobe develops in *Drosophila melanogaster* as a result of cell elongation – individual cells span all the way from the base of the lobe to its tip – and posterior lobe morphology is controlled in part by the apical extracellular matrix (aECM) component Dumpy. We therefore tested whether the aECM also underlies posterior lobe diversification between *Drosophila simulans* and *Drosophila mauritiana*. By labeling the aECM with fluorescent lectins, we have found that it forms attachments to the posterior lobe and other genital structures during early development, and these attachments are more extensive in *Drosophila simulans*, the species with the larger lobe. We also found that the lobe-specific gene expression pattern for *dumpy* is expanded in *Drosophila simulans*, which suggests that these morphological changes are controlled at the level of transcriptional regulation. Finally, we have built cellular simulations of posterior lobe development to investigate the role of the aECM in cellular elongation – whether it exerts an active contractile force on these cells or simply functions as a passive scaffold – and whether that role has changed between species. Our results suggest that morphological diversity may be generated by alterations in extracellular matrix organization during development, and that we can find the genes controlling this process within quantitative trait loci associated with genital evolution.

323A Tracking Natural Variation in Tolerance to Transposable Elements Across Time *Llewellyn Green*, Savana Hadjipanteli, Erin Kelleher The University of Houston

Transposable Elements (TEs) are fragments of selfish DNA that multiply and propagate throughout the genome. These transposition events can lead to DNA damage, which severely impacts the germ-line integrity of the host, and the fitness of their offspring. While the evolution of resistance via the suppression of TE replication has been well characterised, it has been suggested that tolerance— where germ-line fitness is maintained regardless of transposition—could also potentially evolve in the aftermath of a new TE invasion.
The *P*-element, which first appeared in *Drosophila melanogaster* mid-way through the 20th century, is one of the best characterised eukaryotic TEs. As *D. melanogaster* had already been long established as a model organism by the time the *P*-element incursion occured, this particular host/TE system provides us with an excellent opportunity to study potential variation in TE tolerance both pre- and post invasion. Previous quantitative trait locus (QTL) analysis utilizing variation in natural strains lead to the identification of 22 SNPs associated with greater *P*-element tolerance in *bruno*, a gene thought suppresses *P*-element induced ovarian atrophy. As founder strains were all established in research labs prior to the *P*-element invasion, this suggests that the observed variation in tolerance is due to standing variation.
In order to develop a historical picture of TE tolerance over time, we performed whole genome sequencing of a panel of *D. melanogaster* lines collected from different points across the 20th century. We are using these data to determine what alleles were segregating at both the *bruno* locus and number of genes involved in double-strand break (DSB) repair prior to the invasion of the P-element, and how these allele frequencies changed in the aftermath. In addition to characterising TE tolerance, these historical genomes also give us the opportunity to track and compare other variants that have changed in frequency over the same time period due to other selection pressures, such as climate change and insecticide resistance.

324B Intralocus sexual conflict drives new gene evolution in *Drosophila* *Deanna Arsala*[1], Shengqian Xia[1], Shuaibo Han[1,2], Daniel Sanchez[1], Manyuan Long[1] 1) University of Chicago, Chicago, IL; 2) Zhejiang University, Hangzhou, China

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*. We have conducted a CRISPR/Cas9 knockout screen of 36 evolutionarily young, sex-biased gene duplicates in *Drosophila*. These genes have sex-specific patterns of expression—having high levels of expression in the reproductive tissues of either male or female flies. We have partially assessed the function of half of these duplicates and found that loss-of-function mutations in half of these genes confer a significant reduction in either male or female fertility. Bulk and single-cell RNAseq experiments are being employed to identify the cell types and genetic pathways involved in mitigating sexual conflict in the reproductive tissues. Our preliminary analysis suggests that intralocus sexual conflict drives the evolution of new genes with an unexpectedly large proportion contributing to sex-specific fitness.

325C Identifying the epigenetic determinants of gene-by-environment interactions using *Drosophila melanogaster* diapause as a model *Abigail DiVito Evans*, Paul Schmidt, Mia Levine University of Pennsylvania, Philadelphia, PA

Genes and the environment interact to pace developmental transitions. The initiation, cessation, and pausing of such transitions typically depend on genotype-specific responses to environmental cues, i.e. gene-by-environment interactions (GxE). Here we investigate the epigenetic determinants of GxE in the context of developmental pausing. We exploit a seasonally- and geographically- variable reproductive arrest phenotype ("diapause") in *Drosophila melanogaster*. Diapausing females suspend reproduction in response to low temperatures and short days that signal the start of winter. When warm temperatures and long days return in the spring, the arrested ovaries re-initiate egg development. This arrest is accompanied by extensive and coordinated changes in gene expression in the ovary. We hypothesize that epigenetic factors control the diapause gene expression program in the ovary and that this epigenetic control depends on genotype. Consistent with epigenetic regulation, inbred lines that lack genetic variation exhibit incomplete penetrance—only a portion of individuals in the same environment with the same genetic background enter diapause. To identify epigenetic factors that mediate gene expression GxE in diapause, we exploit genetic variation between inbred lines with differing diapause penetrance (a "high penetrance" line with 90% diapause and a "low penetrance" line with 5% diapause). Using Western Blots, we screened histone mark abundance of H3K36me1, H3K27me3, H3K9me3, and H3K4me3 between diapause and age- and temperature- matched reproductive ovaries. We discovered that H3K4me3 is depleted in diapause ovaries, but only in the high penetrance line. We predict that experimentally depleting H3K4me3 in the ovary of the high penetrance line will increase diapause penetrance while experimentally elevating H3K4me3 will decrease diapause penetrance. We predict no such effects in the low penetrance line. We will present the consequences of H3K4me3-depletion and -enrichment in both lines and determine whether H3K4me3-dependent penetrance is genotype-specific. Analyzing RNA-seq reads from these same ovaries, we will delineate those H3K4me3-sensitive genes and pathways that are also differentially regulated by the diapause gene expression program. Together, these data may reveal genotype-dependent epigenetic regulation of GxE that mediate an important adaptive phenotype.

326A Redox balance and the oxidative stress response following acute heat stress of the early embryo in temperate and tropical lines of Drosophila melanogaster *Thomas O'Leary*, Brent Lockwood University of Vermont, Burlington, VT

Early embryos are particularly vulnerable to acute heat stress compared to later life stages. But previous work on Drosophila melanogaster has shown that tropical lines are more heat tolerant than temperate lines, suggesting that selection has made tropical embryos more robust to heat stress. Heat-tolerant genotypes more highly express oxidative stress response genes compared to heat-sensitive genotypes. Beyond the clear benefit of limiting the accumulation of reactive oxygen species and oxidative damage, a robust oxidative stress response may be crucial to maintaining redox balance, which has been shown to be important for progression through early embryogenesis. Here, we characterized redox status and oxidative damage in early embryos (0 – 1 hr after egg laying) following acute heat stress. The response of the embryo was measured through (i) the ratio of redox couples (NADH:NAD+ & GSH:GSSG) as an indication of the general redox state, (ii) superoxide dismutase (SOD) activity to quantify the antioxidant response, and (iii) lipid peroxidation to assess the level of oxidative damage. This work aims to elucidate whether the oxidative stress response is a molecular mechanism underlying acute heat tolerance in early embryos, and therefore, whether maintaining redox homeostasis is critical to heat tolerance in D. melanogaster embryos.

327B Widespread effects of early embryonic thermal stress on morphology, physiology and performance across the lifespan in *D. melanogaster* *Sara Helms Cahan*, Andrew Stoloff, Katie Bora, Collin Brown, Caela Flanagan University of Vermont

Environmental temperature has profound effects on every aspect of the development and performance of poikilothermic

organisms, and thermal limits play an important role in setting species geographic distributions. The most obvious effect of temperature extremes is mortality, which is used to determine the extent of the thermal safety margin, the difference between environmental temperatures and the thermal limits of the organism. However, significant negative impacts on performance may occur well below the LT_{50} value, particularly for thermally sensitive life stages. To investigate the importance of sublethal effects, we compared multiple aspects of survival, development and thermal performance of developing *D. melanogaster* Canton-S eggs or larvae exposed to unstressed or moderately stressful low and high developmental temperatures (18°C, 25°C and 30°C) after one hour, 24 hours, or 60 hours post-laying. One-hour embryos displayed widespread deficits that were largely absent in flies transferred later in development, with somewhat different elements of performance impaired under cold and heat stress. Adult-to-pupal survival of cold-stressed early embryos was 70% of survival in unstressed conditions but was not reduced at 30°C; however, heat-stressed embryos suffered a 50% mortality rate during the pupal stage, and of those that emerged successfully, 35% displayed a crumpled-wing phenotype that rendered them unable to fly, and 30% showed significant locomotion deficits in an adult climbing assay. Adult thermal performance was also impacted, with both cold-stressed and heat-stressed early embryos displaying a ~0.3°C reduction in critical thermal maximum, while cold-stressed embryos showed substantially reduced capacity to improve their critical thermal minimum through developmental acclimation. Altogether, these delayed effects of early thermal stress summed across the lifespan have the potential to reduce mean fitness below levels needed for population replacement, which may have important implications for prediction of population persistence under projected climate change.

328C Ultra Violet radiation tolerance between *Drosophila* species from São Tomé and Africa: Adaptation across *Drosophila yakuba* and *Drosophila santomea* *James Titus-McQuillan*, Rebekah Rogers UNC Charlotte

The genetic basis of phenotypic differences between species is among the most longstanding questions in evolutionary biology. How new genes form and the processes selection acts to produce differences across species are fundamental to understand how species persist and evolve in an ever-changing environment. Adaptation and genetic innovation arise in the genome by a variety of sources. Functional genomics requires both intrinsic genetic discoveries, as well as empirical testing to observe adaptation between lineages. Here we explore two species of *Drosophila* on the island of São Tomé and mainland Africa, *D. santomea* and *D. yakuba*. These two species both inhabit the island, but occupy differing species distributions based on elevation, with *D. yakuba* also having populations on mainland Africa. Intrinsic evidence shows genes between species may have a role in adaptation to higher UV tolerance with DNA repair mechanisms (*PARP*) and resistance to humeral stress lethal effects (Victoria). We conducted empirical assays between island *D. santomea*, *D. yakuba*, and mainland *D. yakuba*. Flies were shocked with UV B radiation (@ 302 nm) at 1650-1990 mW/cm^2 for 30 minutes on a transilluminator apparatus. Custom 5-wall acrylic enclosures were constructed for viewing and containment of flies. All assays were filmed for reproducibility. Island groups did not show significant differences between fall-time under UV stress and recovery time post-UV stress test. Preliminary evidence shows mainland flies are less resistant to UV radiation than their island counterparts. Further work exploring the genetic basis for UV tolerance will be conducted from empirical assays. Understanding the mechanisms and processes that promote adaptation and testing extrinsic traits within the context of the genome is crucially important to understand evolutionary machinery.

329A Genomic Benchmarks: A Collection of Datasets For DNA Sequence Classification *Petr Simecek*, Katarina Gresova, Vlastimil Martinek, David Cechak, Panagiotis Alexiou Central European Institute of Technology, Masaryk University

Recently, deep neural network models have been successfully applied to identify functional elements in the genomes of D. melanogaster and other organisms, e.g., promoters [1], chromatin folding [2], splice sites [3]... Unfortunately, it is not easy to compare the quality of these methods since they use different data preprocessing approaches. In other fields, benchmarks datasets have been established as a gold standard for comparison, e.g., ImageNet for image recognition, IMDB Sentiment for text classification, SQuAD for question answering, and CASP data for protein folding prediction [4].

We are proposing a collection of datasets that may serve as a benchmark for the classification of genomic sequences. The collection is distributed as a Python package ‘genomic-benchmarks’ that is distributed through The Python Package Index (PyPI). Each dataset is stored both as a list of genomic interval coordinates and DNA sequences. The package provides utilities for conversion between these two formats, data cleaning procedures and checks. Furthermore, it contains functions that make the training of a neural network classifier easier, like PyTorch and TensorFlow data loaders. We hope other researchers will use our datasets to evaluate the quality of their algorithms.

The package ‘genomic-benchmarks’ and demo notebooks on how to use it are available on GitHub:

https://github.com/ML-Bioinfo-CEITEC/genomic_benchmarks

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330B Insights into *D. melanogaster* and *D. simulans* transcriptome evolution and complexity using transcript distance (*TranD*) Adalena Nanni[1,2], James Titus-McQuillan[3], Oleksandr Moskalenko[4], Francisco Pardo-Palacios[5], Sarah Signor[6], Srna Vlaho[7], Zihao Liu[1,2], Ana Conesa[2,5,8], Rebekah Rogers[3], *Lauren McIntyre*[1,2] 1) Department of Molecular Genetics and Microbiology, University of Florida, Gainesville, FL; 2) University of Florida Genetics Institute, University of Florida, Gainesville, FL; 3) University of North Carolina Department of Bioinformatics, Charlotte, NC; 4) University of Florida Research Computing, University of Florida, Gainesville, FL; 5) Dept. of Applied Statistics and Operational Research, and Quality, Polytechnical University of Valencia, Spain; 6) Department of Biological Sciences, North Dakota State University, Fargo, ND; 7) Department of Biological Sciences, University of Southern California, Los Angeles, CA; 8) Institute for Integrative Systems Biology, Spanish National Research Council (CSIC), Paterna, Spain

Alternative splicing is an important driver of phenotypic diversity in higher eukaryotes. Understanding how alternative splicing and variation in transcript structure diverge across species can provide insights into phenotypic divergence and speciation. Long-read sequencing of mRNA provides an opportunity to observe transcript structure. We present metrics of complexity and nucleotide-level descriptions of structural phenotypes that can be calculated within an individual transcriptome or compared across transcriptomes. Using this tool, we show how patterns of transcriptome complexity can be compared across species without depending on the identification of orthologs. We further demonstrate that distance metrics can be used to compare transcriptomes of the closely related species, *D. melanogaster* and *D. simulans*, and identify novel exons which we validate. We implement out metrics in a PyPi package *TranD* and in the open source bioinformatics for everyone platform Galaxy (www.Galaxyproject.org) which will empower a wide range of researchers to quickly identify minimum distance transcripts between species, interesting structural variants within species and genome complexity enabling deeper understanding of splicing mechanisms and transcript evolution.

331C Bacterial infection promotes transposable element activation in *Drosophila* species *Sabrina Mostoufi*, Nadia Singh University of Oregon, Eugene, OR

Transposable elements (TEs) are short, repetitive sections of ancient viral DNA capable of inserting themselves into new parts of a genome. This ability has allowed TEs to invade the genomes of nearly all organisms, where they make up 5-75% of the genomes of plants, animals, and bacteria. TE activation can also affect gene expression, mutation, and recombination, making them powerful contributors to the evolutionary process. Stressful stimuli can cause TE activation to increase, creating another link between TEs and evolution. Infection can be a particularly stressful event for organisms, leading to a cascade of gene expression changes. Several studies have illustrated that infection can cause increased TE expression, but it is unclear how these processes differ between host species and pathogen species. Here we test the hypothesis that infection causes elevated TE expression in *Drosophila* species and investigate how pathogen species influences that expression. We analyzed several RNA-seq datasets of varying *Drosophila* species, infection types (bacterial, fungal, viral), and tissue types. Our results find that infection status is significantly associated with TE expression, but that the magnitude of that effect depends on host species and infection type.

332A Prevalence of galbut virus in wild Drosophila melanogaster populations and to lab colonization *Tillie Dunham*, Mark Stenglein Center for Vector-Borne and Infectious Disease, Department of Microbiology, Immunology, and Pathology, Colorado State University, Fort Collins, CO, USA

Galbut virus is remarkably common in wild *Drosophila melanogaster*. Every population that has been tested contains some infected individuals. But on average only 60% of flies are infected and there is evidence that some flies are resistant to infection. To better understand galbut virus-host interactions in wild and lab-reared populations, we pursued three avenues of investigation. First, we quantified galbut virus prevalence in wild populations sampled from different geographic locations over 5 years. We found that galbut virus prevalence varied widely from 0-96%, even in populations sampled from nearby geographic locations. We also found that galbut virus load in individual flies can be binned into 3 phenotypes: high positives, with galbut virus RNA levels exceeding that of an abundant host mRNA, low positives, with galbut virus RNA levels >100,000x lower, and negatives, flies with undetectable infection. Second, we performed an experiment to assess whether galbut virus prevalence would change over time in multiple independent wild-caught populations reared in lab. This will allow us to assess possible fitness costs to infection by tracking changes in infection frequency from generation to generation. Finally, to better understand the low positive phenotype, we bleached eggs then placed individual eggs into vials to be reared to adulthood. We did this in parallel with unbleached eggs that were reared together to have a baseline comparison. This experiment will allow us to determine whether the low positive infection phenotype represents legitimate low-level infection or contamination from highly infected flies. Overall, these

studies contribute to a fuller understanding of the variability associated with infection by this exceptionally common persistent *Drosophila* virus.

333V The role of chromatin and DNA sequence changes in *de novo* gene origin *Logan Blair*, Julie Cridland , David Begun, Artyom Kopp UC Davis

Although it's clear that some genes originate *de novo* from nongenic sequence, the molecular mechanisms through which they first gain expression remain unclear. One hypothesis is that novel transcripts evolve more easily in regions with ancestrally permissive chromatin structure. Alternatively, *de novo* genes may evolve from ancestrally inaccessible regions and the transition to open chromatin is critical to *de novo* gene origin. To distinguish between these models, I used ATAC-seq to examine chromatin accessibility around recently evolved *de novo* transcripts. I compared chromatin states of accessory gland tissue between five *Drosophila melanogaster* strains polymorphic for *de novo* transcript expression, as well as two closely-related species in which orthologous DNA sequences are not expressed. I found that open chromatin in *de novo* transcript promoters was frequently not exclusive to genotypes in which the *de novo* transcript was expressed. However, the magnitude of chromatin accessibility was greater in lines expressing *de novo* transcript, and much greater than that of orthologous regions in related species. Next, to determine the location of *cis*-regulatory variants associated with *de novo* transcript expression, I generated RNA-seq libraries from accessory gland tissue in 29 *D. melanogaster* strains. I found that SNPs associated with *de novo* transcript expression frequently occur outside the regions of ATAC-seq peaks. Together these results paint a complex picture of *de novo* gene origin in which an increase in chromatin accessibility is required but frequently occurs in ancestrally open regions.

334V Experimental Evolution for Longevity Differentiation in *Drosophila melanogaster* *Karen Walsh*[1], Parvin Shahrestani[1], Molly Burke[2] 1) Cal State University, Fullerton; 2) Oregon State University

The evolutionary theory of aging proposes that the forces of natural selection start to decline after the first age of reproduction and continue to steadily decline until the last age of reproduction, where these forces stabilize at zero or become negligible. Past studies have used *Drosophila melanogaster* populations to show that gradually postponing the first age of reproduction, postpones the age at which the forces of natural selection begin to drop, and results in delayed aging and increased longevity. However, genomic studies involving longevity remain underpowered due to limitations in replication. Long-lived populations were created by progressively postponing the first age of reproduction from 14 days to 70 days. To maximize the statistical power of finding these candidate genes, we used ten replicate populations selected for increased longevity, maintained on 70-day discrete generation cycles, and ten replicate populations that are treated as matched controls, maintained on 14-day discrete generation cycles. We have completed twenty generations of laboratory selection for postponed reproduction. Populations selected for delayed reproduction had increased longevity, increased fecundity, increased desiccation and starvation resistance, and increased wet and dry body weight. Samples from these populations have been frozen/preserved for each generation of selection. We next aim to identify candidate genes involved in longevity using a genome-wide analysis of experimentally evolved short-lived and long-lived populations of *D. melanogaster*. Identifying regions of the genome that are differentiated between short and long-lived populations may provide candidate genes for human longevity.

335V Evolution of longevity and immunity differentiation in *Drosophila melanogaster* *Joshua Glowalla*[1], Molly Burke[2], Parvin Shahrestani[1] 1) California State University, Fullerton, CA; 2) Oregon State University, Corvallis, Oregon

Theoretically a result of declining forces of natural selection, aging is a decline in fitness characters. Previous research has established *Drosophila melanogaster* as a model system to study the evolution of aging. Investigations utilizing experimental evolution have revealed a few well-defined relationships between aging and other traits, but the evolutionary relationship between longevity and immune defense remains unclear. While some studies suggest an evolutionary trade-off between these traits, others suggest positive correlations. Through a 10-fold replicated experimental evolution study, we have created populations that are diverged in age-of-reproduction. Late reproducing populations have evolved increased longevity and also improved immunity against the fungal pathogen *Beauveria bassiana*. We will test these populations for divergence in immunity against other pathogens. Analyses of whole-genome sequences throughout the selection process will provide information both about causative alleles and about their trajectories of change. Moreover, whole-genome comparison of our populations will reveal genetic connections between longevity and immunity. Advances in healthcare and technology have reduced the impact of many diseases on human populations, but consequently the percentage of people living to late age have increased along with the number of people with age-related illnesses. Aging and associated changes to immunity have become global health concerns which are best understood in the context of evolution.

336V Trade-offs between cost of ingestion and rate of intake drive defensive toxin use *Tyler Douglas*[1], Sofia Beskid[3], Callie Gernand[2], Brianna Nirtaut[2], Kristen Tamsil[1], Richard Fitch[2], Rebecca Tarvin[1] 1) University of California Berkeley, Berkeley, CA; 2) Indiana State University, Terre Haute, IN; 3) University of Texas at Austin, Austin TX

Animals that ingest toxins can become unpalatable and even toxic to predators and parasites through toxin sequestration. Because most animals rapidly eliminate toxins to survive their ingestion, it is unclear how populations transition from susceptibility and toxin elimination to tolerance and accumulation as chemical defense emerges. Studies of chemical defense have generally focused on species with active toxin sequestration and target-site insensitivity mutations or toxin-binding proteins that permit survival without necessitating toxin elimination. Here, we investigate whether animals that presumably rely on toxin elimination for survival can utilize ingested toxins for defense. We use the A4 and A3 *Drosophila melanogaster* fly strains from the Drosophila Synthetic Population Resource (DSPR), which respectively possess elevated and reduced metabolic nicotine resistance amongst DSPR fly lines. We find that ingesting nicotine increased A4 but not A3 fly survival against *Leptopilina heterotoma* wasp parasitism. Further, we find that despite possessing genetic variants that enhance toxin elimination, A4 flies accrued more nicotine than A3 individuals likely by consuming more media. Our results suggest that enhanced toxin metabolism can allow for greater toxin intake by offsetting the cost of toxin ingestion. Passive toxin accumulation that accompanies increased toxin intake may underlie the early origins of chemical defense.

337V Dietary utilization drives the differentiation of gut bacterial communities *Chau-Ti Ting*[1], Jia-Syuan Chen[1], Shu Fang[2] 1) National Taiwan University, Taipei, Taiwan; 2) Academia Sinica, Taipei, Taiwan

Gut bacteria have been suggested to play vital roles in the dietary detoxification, digestion, and nutrient supplementation of hosts during dietary specialization. The roles of gut bacteria can be revealed by comparing bacterial communities between specialist and generalist hosts. However, it is often difficult to determine whether bacterial community differentiation is due to host dietary adaptation or divergence. To address this question, we investigated the bacterial communities from two Araceae-feeding Colocasiomyia species and further performed a meta-analysis by incorporating the published data from Drosophila bacterial community studies. By comparing three types of specialists (Araceae-feeding, mycophagous, and cactophilic) with generalist flies, we detected the structural and functional differentiation of the bacterial communities between specialists and generalists. The structure differentiation showed that Bacteroidetes and Firmicutes inhabited specialists, while more Proteobacteria inhabited generalists. The functional differentiation revealed that amino acid and energy metabolic pathways were overrepresented in specialists and generalists, respectively. This differentiation is associated with the higher utilization of structural complex carbohydrates, utilization of proteins, demand for vitamin B12, and demand for detoxification in specialists than in generalists. These results reveal that the interaction of bacterial communities and hosts might consequently facilitate the dietary specialization and ecological adaptation of animals.

338V Identification of a pseudogene derived from *Arr1* in *D. ananassae* *Ishtar Olaveja*[1], W. Hayden Kee[2], Evan Merkhofer[3], Don Paetkau [4], Wilson Leung [5], Cindy Arrigo[1] 1) New Jersey City University, Jersey City, NJ; 2) California State University Stanislaus, Turlock, CA; 3) Mount Saint Mary College, Newburgh, NY; 4) Saint Mary›s College, Notre Dame, IN; 5) Washington University in St. Louis, St. Louis County, MO

To facilitate investigations into the expansion of the *Drosophila ananassae* Muller F Element (~19.1 Mb) compared to *Drosophila melanogaster* (~1.3 Mb), we are manually annotating the protein-coding genes located on the *D. ananassae* F Element and a ~1.8 Mb region from the *D. ananassae* Muller D Element. As part of this analysis, we identified a region within scaffold QMES02000178 of the *D. ananassae* DanaRS2 genome assembly which shows significant sequence similarity to the *Arr1* gene in *D. melanogaster.* Because past studies have shown that more than 90% of the genes remain on the same Muller Element across different *Drosophila* species, the *D. ananassae* scaffold QMES02000178 has been tentatively assigned to the D Element based on the protein-coding genes surrounding this *Arr1* feature (i.e., *CG14109*, *CG10725*, *CG10154*, *CG10713*, *CG10222*, *CG33263*, *flr*). In contrast, the *Arr1* gene is located on the Muller B Element (chromosome 2L) in *D. melanogaster*. Analysis based on the evidence provided by computational gene predictors (e.g., genBlastG, GeMoMa, Genscan, Geneid, and Augustus), RNA-seq data, and FlyBase BLASTP search results placed the putative ortholog of *Arr1* on scaffold QMES02000007 in the *D. ananassae* DanaRS2 assembly. The *D. ananassae* scaffold QMES02000007 has been tentatively assigned to the B Element, suggesting that synteny for Arr1 is preserved between *D. melanogaster* and *D. ananassae*. The BLASTX alignments of the genomic regions surrounding the feature on the *D. ananassae* scaffold QMES02000178 and the protein sequence for *D. melanogaster* Arr1-PA include multiple frame shifts and in-frame stop codons, thereby supporting the hypothesis that the feature on QMES02000178 is a pseudogene derived from *Arr1*. The putative *Arr1* pseudogene is also flanked by the DNA2-1_DAn and But2 DNA transposons. Future studies will identify and annotate additional pseudogenes located on the *D. ananassae* F Element and on the euchromatic reference region from the D Element, in order to assess the impact of the expansion of the *D. ananassae* F Element on the frequency of pseudogenization.

339V Frequent co-domestication of *PIF-like* transposable element proteins in insects *Fatema ruma*[1], Dragomira N. Markova[1], Claudio Casola[2], Ayda Mirsalehi[1], Esther Betran[1] 1) University of Texas at Arlington; 2) Texas A&M University

Transposable elements (TEs) are genetic units that move and amplify within a host genome. In recent years, an increasing number of studies have shown that one of the most direct contributions of TEs to their host is through the process of

'molecular domestication' whereby the genes normally encoded by and serving the replication of a TE are co-opted by the host genome to create new gene(s) with cellular function. Thus, TE proteins are an important contributor to the emergence of novel host proteins. Despite the relative abundance of RNA TEs in eukaryotic genomes, DNA TE proteins and most notably their transposase, responsible for the excision and movement of the elements within the host genome, are generally considered to be more likely to be co-opted by the host than any other TE-derived protein. We have been studying four domesticated transposases from the *PIF/Harbinger* DNA family of TEs in *Drosophila melanogaster*, named ***D****rosophila* ***P****IF* ***L****ike* ***G****enes* (*DPLGs*). So far, all *PIF* transposable elements known in plants and animals distinguish themselves from traditional DNA transposons by the presence of two independent transcription units. One encodes a protein representing the catalytic transposase, while the other encodes a protein with a MADF domain. We hypothesize that MADF proteins, a big gene family of regulatory proteins in *D. melanogaster*, to be derived from the same transposable elements. We also hypothesize that there should often be co-domestications of transposase and MADF proteins because the transposase translocates to the nucleus by the MADF protein. This is true for *HARBI1* and *NAIF1* in humans, *DPLG7* and *DPM7* in *Drosophila* and two co-domestications in *Arabidopsis* (*ALP1* and *ALP2* and *HPD1* and *HPD2*). To provide further support to this co-domestication model, we investigated numerous insect species genomes for additional evidence of *PIF* TE domestication events and explore the co-domestication of the MADF protein from the same TE insertion. We present evidence of at least five *PIF* TE domestication events in insects: two co-domestication of both transposase and MADF proteins in *Anopheles* (Diptera) and one transposase only domestication event and one co-domestication event Lepidoptera, and one transposase only domestication event in cockroaches (Blattodeae). Thus, our results show that domestication of *PIF* transposases is frequently accompanied by the co-domestication of a cognate MADF protein potentially for regulatory functions further supporting the common origin of both PIF proteins.

340V Evolutionary diversification and repeated gene capture by telomeric retrotransposons across the Drosophila genus *Jae Hak Son*[1], Mia Levin[2], Christopher Ellison[1] 1) Department of Genetics, Human Genetics Institute of New Jersey, Rutgers, The State University of New Jersey, Piscataway, New Jersey, United States of America; 2) Department of Biology and Epigenetics Institute, University of Pennsylvania, Philadelphia, United States of America

Transposable elements (TEs) are mobile genetic elements that must replicate faster than their host to avoid extinction. TEs often evolve antagonistically with their host in a classic evolutionary arms race scenario. 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 traditionally 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 a mutualism. Furthermore, we recently found that the *D. melanogaster TART-A* telomere-specialized transposon has captured a portion of the piRNA pathway gene, *nxf2*, which allows it to target *nxf2* for suppression, again consistent with antagonistic evolution. Here we have examined the evolutionary diversification of telomere-specialized retrotransposons across the *Drosophila* genus using publicly available long-read genome assemblies from 106 *Drosophila* species genomes. We identify 9 major telomeric retrotransposon clades from the *gag* gene and 7 major clades from the *pol* gene, including the previously described HTT clade from *melanogaster* group and the TR2 clade found in the *ananassae* and *montium* groups. We find that host gene capture among these telomere-specialized TEs is relatively common and propose that this phenomenon may serve as an important source of host gene regulation in the *Drosophila* germline.

341V Analysis of eIF4E1 Conservation and Synteny across Drosophila Species to Understand the Evolution of the Insulin Pathway *Jessica Strand*, Jami Feist, Emma Sirjord, Sydney Payeur, Breanna Hoffman, Paula Croonquist Anoka Ramsey Community College

The Insulin/Tor signaling pathway regulates important physiological functions such glucose and lipid metabolism, cell growth and survival. It is highly conserved from fruit flies to mammals. This pathway is also critical for homeostasis and its dysregulation results in prevalent human diseases such as in Type II Diabetes, Cardiovascular disease and even cancer. The eukaryotic translation initiation factor 4E1 (eIF4E1) encodes for a member of the eIF4F cap-binding complex that is required for cap-dependent translation of mRNA. The purpose of this study was to determine the conservation of the eIF4E1 gene in multiple species of Drosophila relative to *D. melanogaster*. It was hypothesized that the species highly divergent from the reference species would exhibit less eIF4E1 conservation than those closely related to the reference species. It was predicted that this gene would be most conserved in *D. serrata* and least conserved in *D. arizonae*. Gene models were proposed utilizing the UCSC Genome Browser, BLAST, Flybase gene record finder and verified by Gene Model Checker. The synteny analysis and protein alignments observed suggest that eIF4E1 in *D. takahashii* is the most conserved and D. arizonae is the most divergent. However, all five species had a high eIF4E1 protein identity ranging 81-94%. Previous evidence has demonstrated that position in the insulin signaling pathway may affect selective constraint, with downstream genes evolving at a slower rate than genes closer to the membrane. This may explain the high level of conservation for the eIF4E1 gene. Further studies should aim to investigate the impact of position, connectivity and/or

other factors shown to influence this pathway's selective constraint.

342V Genome-wide relaxation and phylogenetic inertia of codon usage bias in the Neotropical *Drosophila saltans* species group *Carolina Prediger*[1,2], Erina A. Ferreira[2], David Ogereau[2], Amir Yassin[2], Lilian Madi-Ravazzi[2] 1) São Paulo State University (UNESP), Institute of Biosciences, Humanities and Exact Sciences, São José do Rio Preto, São Paulo, Brazil; 2) Laboratoire Evolution, Génomes, Comportement, Ecologie (EGCE), CNRS, IRD, Université Paris-Saclay, Gif-sur-Yvette, France

It is well established that *Drosophila* flies favor codons ending in C|G but relaxation in codon usage bias (CUB) pattern was reported for the *willistoni* and *saltans* groups, two Neotropical sister clades of the subgenus *Sophophora*. Those reports were, however, carried out with few genes (*saltans* group) or single genome (*D. willistoni*). Here, we generated whole genome sequences for 15 out of 23 species of the *saltans* group and evaluated CUB using a large set of genes in terms of the effective number of codons (ENC) and the relative synonymous codon usage (RSCU) index. We also investigated three forces that may be contributing to CUB, namely, mutational bias, selection pressure and drift. Our results indicated relaxation in CUB (mean ENC=48.33). Average RSCU index showed preference for codons ending in A|T. To evaluate mutational bias strength, we found moderate positive correlation between the RSCU of codons ending in G|C and the average G|C content at third position (GC3) for a given gene and a similarly moderate but negative correlation was seen for codons ending in A|T. Regarding translational selection, we found no correlation between tRNA adaptation index (stAI) and ENC after estimating tRNA diversity and content for four species for which genome assemblies have recently been published. We verified the strength of drift, by estimating the phylogenetic signal of RSCU on a phylogeny inferred from 30 genes, a heatmap with a hierarchical cluster analysis and a correspondence analysis. The first method indicated very strong phylogenetic signal for RSCU in the *saltans* group that was largely confirmed by the phylogenetic clustering of species by the second and third methods. In conclusion, our results demonstrate that relaxation of CUB in the *saltans* group is a general genome-wide pattern that has likely originated by mutational bias in the ancestor of the *willistoni* and *saltans* groups, and has since drifted along the evolutionary history of *saltans* species.

343V Resemblances Amomg Different Romanian Ecotypes of Drosophila melanogaster L. *Gallia Butnaru*[1], Ioan Sarac[2] 1) Banat University of Agricultural Sciences and Veterinary Medicine Regele Mihai I al României from Timişoara, România; Department of Genetics; 2) Banat University of Agricultural Sciences and Veterinary Medicine Regele Mihai I al României from Timişoara, România

19 *Drosophila* Romanian ecotypes were notice compared with 3 standard genotypes. The ecotypes were collected from geographically differentiated areas and anthropogenic polluted. The aim was to establish the relationship between the ecotypes evolved in the western, central and eastern areas of România. The multivariate analysis of variance revealed the significant differences between individuals [male/female] and among ecotypes. The male body average size represented 82.71% (2.92±0.05mm) compared to the female average size (3.53±0.06 mm). The variability of body size varied from 2.93 to 4.60mm on female and 2.43 to 3.83mm on males. Among female the difference of size averages was larger than among males (d_F=1.67mm>d_M=1.4mm). Even in this circumstance the male size varied from 80.2% to 84.0% at the same size of females. The results indicated that only 36.4% and 45.6% female and male, respectively, revealed a larger body size than the overall average size. It was found at 81.8% of ecotypes the influence of environment was small and insignificant. The multivariate analysis of variance regarding the participation of females and males at the general body size average of the ecotypes pointed out the population significant involvement of Black Plopşoru and Urdari (A and B) from Gorj County and Ebony, Timişoara-N, Şag (C) from Timiş County (LSD5% = 0.31 mm).
It was concluded that knowledge of quantitative traits, in our case the body size, is directly associated with adaptability to extreme conditions such as anthropogenic pollution and limited motility.
Even if the fruit fly populations are not real isolated it has been identified inter-populations and intra-population phenotypic polymorphism. The presence different forms of *Drosophila* in the same niche Plopsoru village [Black Plopşoru (big and normal) and Grey Plopşoru] and in Roşia Montana [(Big and normal)] pointed out their commune origin separated due to disruptive selection. Only at Black Plopşoru the reproductive barrier was prove. This analysis demonstrated that temperature conditions, both within and between generations, influence territorial success of flies [*Zamudio* et al. 1995].

344V Screening for cryptic genetic variation in natural populations of *Drosophila melanogaster* *Gabriella Moreno*, Nicolette Alvandian, Alexis Long, Emily Jabourian, Lukas Prelooker, David Marcey California Lutheran University

Cryptic genetic variation may be an important component of adaptive, deleterious, or neutral variation that is contingent upon environmental or genetic circumstances to be expressed. We are conducting screens for inducible, phenotypic variation in natural populations of *Drosophila melanogaster*. The screen is based on a model for the production of head defects by the *extra eye* mutation (*ee*), which is incompletely penetrant, variably expressed, and conditionally dominant. The proposed epigenetic model predicts that new mutant phenotypes may be uncovered in crosses between *ee* lines and flies from natural populations that harbor P-elements at various genomic locations. Such novel mutants are predicted to be incompletely penetrant and variably expressed, caused by epigenetic silencing of genes residing at genomic

positions near P transposable elements. In an initial study described here, we conducted a screen for such mutations by scoring flies in lines established from crosses of 40 separate, wild-derived lines from various geographic coordinates to *ee*. In several such lines, new mutations were recovered that exhibit epigenetic properties. The induction of these mutant phenotypes by crosses to *ee* was repeatable and not observed in control lines, suggesting that they are not induced *de novo*, but rather represent cryptic phenotypes induced by genetic elements in the *ee* line. *Cby* (crybaby) and *Bby* (Beady), both affecting ventral eye development, were observed in lines derived from Brazil and Australia, respectively. *Tby* (*tumorous baby*), obtained independently in a second line derived from Brazil, yields melanotic tumours in ~3-5% of 3rd instar larvae. Our results to date suggest that variation in natural populations may include cryptic, epigenetic sources, which are revealed under particular genetic contingencies. Screens of multiple, wild strains for additional examples of cryptic phenotypes are ongoing, as are genetic and molecular characterizations of mutant phenotypes so far recovered.

345V Genotype-dependent effects of human disturbance on organismal fitness *Heidi Johnson*, Nicole Riddle University of Alabama at Birmingham

Human disturbance causes significant changes to home range and movement distance in wild populations. These changes and other behavior modifications often negatively impact body condition and/or fitness. While disturbance in wild populations is well-documented, there is a lack of systematic investigations defining the impact of the disturbance on energetic tradeoff and body composition. To address this need, we will utilize tools developed to study exercise in Drosophila to mimic disturbance. The Treadwheel induces movement in Drosophila through rotation of the enclosures. To sample genetic variation, we are using strains from the Drosophila Genetic Reference Panel (DGRP). The DGRP is a set of inbred strains derived from wildtype genotypes that models naturally occurring population variation. The DGRP strains differ in baseline activity approximately 525-fold for males and 110-fold for females. They also differ in their weight (1.7-fold), and quantitative magnetic resonance analysis revealed significant variation in body composition among the lines. Differences in weight, activity, and body composition is sex- and genotype-dependent. We use this diverse collection of Drosophila strains to probe the consequences of disturbance on movement patterns, body condition, and organismal fitness. We compare the effects of short-term (5 days of daily treatment on the Treadwheel) and long-term (15 days of daily treatment on the Treadwheel) disturbance regimes. Specifically, we investigate how disturbance alters longevity, offspring count, and physical condition and quantify the impact on organismal fitness, in experiments that are in progress. We expect that the impact of long-term disturbance to be more severe than the impact of short-term disturbance. We also expect to find variation in the responses to the disturbance for all outcome measures and that the outcomes are sex- and genotype-dependent.

346V Evolutionary conservation and divergence of 3D genome organization in Drosophila *Nicole Torosin*, Weihuan Cao, Christopher Ellison Rutgers University

Topologically associating domains (TADs) are 3D organizational units of chromatin that are believed to regulate gene expression by constraining enhancer/promoter interactions. Several early studies found that TADs are highly conserved in both vertebrates and in Drosophila. However, more recent research suggests that TADs may diverge rapidly and that their reorganization is not associated with widespread divergence in gene expression, in contradiction to their supposed role in constraining enhancer/promoter contacts. In this study, we use a comparative genomics approach to estimate the rate of TAD evolution and determine whether the evolutionary conservation of TAD structures is associated with conservation of gene expression. We generated Hi-C chromosome conformation capture data for eleven Drosophila species from the melanogaster group, diverging between 4-32 million years ago. We used a phylogenetic approach to estimate the rate of TAD evolution and found that TADs evolve roughly 10-20-fold faster than other genomic features in Drosophila, such as gene duplicates and chromosomal rearrangements. Next, we found that highly conserved TADs are enriched for the BLACK and BLUE chromatin states which contain Polycomb-repressed and developmentally-regulated genes. These TADs evolve at a significantly slower rate compared to other TADs and show significantly higher constraint in gene expression levels. On the other hand, TADs that are enriched for the YELLOW chromatin state, which contains broadly-expressed, transcriptionally-active genes, evolve faster compared to all TAD domains and show less gene expression constraint. After controlling for chromatin state, we do not find a significant relationship between TAD conservation and interspecies variation in gene expression levels. These results suggest that, in general, most TADs evolve rapidly and their divergence has little effect on gene expression. However, the higher levels of evolutionary conservation and gene expression constraints in TADs enriched for developmentally-regulated chromatin suggests that these TAD subtypes may be more important for regulating gene expression, likely due to the larger number of long-distance enhancer promoter contacts associated with developmental genes.

347V The interaction between male courtship plasticity and female mate choice in *Drosophila melanogaster* *Samuel Marston*[1], Dean Castillo[1,2] 1) University of Utah, SLC, UT; 2) University of Nebraska at Omaha, Omaha, NE

Sexual selection drives rapid speciation by creating divergence in sexual signals and preferences. Geographically isolated populations of *D. melanogaster* exhibit strong asymmetric behavioral isolation and divergence in male courtship

behavior. These lineages are often classified into Z-type lineages from Southern Africa and M-type lineages that occur outside of Africa. Z-type females almost exclusively choose Z-type males over M-type males in mate choice assays. Both Z-type and M-type males adjust their courtship behavior depending on the genotype of the female they are courting. This behavioral plasticity could potentially optimize male mating success when encountering diverse females. However, the mechanism responsible for triggering a plastic behavioral response is unknown. Males might respond to female behavioral feedback or female chemical cues. To determine which female trait facilitates male courtship plasticity we quantified courtship behaviors, focusing on the time spent singing, for males interacting with females wherein we have genetically manipulated their cuticular hydrocarbon (CHC) profile. Using a Z-type male strain that exhibits strong plasticity, we quantified the time spent singing when interacting with an M-type female and a Z-type female to establish baseline plasticity. We then tested these males with the Z-type females in which the coding sequence of the gene *desat2* was replaced using CRISPR*,* creating a null mutant. The gene *desat2* contributes to the production of the CHC 5,9-heptacosadiene (5,9-HD) a compound that is characteristic of Z-type females. This CHC is an isomer of the CHC 7,11 HD which is the most abundant CHC in M-type (non-African) females. Our Z-*desat2* null strain has a CHC profile resembling an M-type female but in choice tests behaves like a Z-type female. Thus we believe this genotype would provide an M-type chemical cue to a male but Z-type like behavioral feedback. We hypothesized that if males use these heptacosadiene isomers to adjust their courtship we would expect to see male courtship of Z-*desat2* null females resemble courtship of M-type females. This experiment will determine which cue triggers male courtship plasticity and how this might contribute to the evolution of reproductive isolation, furthering our understanding of the co-evolution of male behavior and female preference of closely related populations.

348V A locus affecting pigmentation evolution and male mating success between two sibling species in *Drosophila* Jean David, Erina Ferreira, Laure Jabaud, David Ogereau, Héloïse Bastide, *Amir Yassin* Laboratoire Évolution, Génomes, Comportement et Écologie, CNRS, IRD, Université Paris-Saclay, Gif-sur-Yvette, France

New species originate when diverging populations become reproductively isolated and morphologically distinct. In theory, genetic correlation between the two phenotypes through pleiotropy or partial linkage significantly accelerates speciation. Here, we investigate the genetic correlation between mate choice and pigmentation difference in a pair of sibling species, *Drosophila yakuba* and *D. santomea*. We introgressed the "light" phenotype of *D. santomea* into the "dark" background of *D. yakuba* through a series of backcrosses. Two introgressed "light"*yakuba* strains that slightly differed in the degree of pigmentation were obtained. Genome sequencing revealed that the darker of the two strains had two introgressed X-linked loci, each centered on a melanin-synthesis gene, namely *yellow* and *tan*. The lighter introgressed strain had, in addition to the two X-linked loci, a third introgressed locus on autosomal arm 3L. The third locus centered on the transcription factor *Grunge*, which regulates the expression of the melanin-synthesis genes *ebony* and *tan* in *D. melanogaster*. We then conducted reproductive isolation assays between the two introgressed strains and strains from the parental species, measuring male and female mating latency and choice. We found no evidence for reproductive isolation in the darker introgressed strain, in contrast to the lighter strain, which exhibited decreased male mating success most likely due to increased latency. Those findings indicate that reproductive isolation and pigmentation difference between *D. yakuba* and *D. santomea* are partly linked through a single autosomal locus. Future dissection of this locus will elucidate the molecular underpinnings of such partial correlation in this primary model of speciation research.

349V Evidence of horizontal transmission of *Wolbachia* in *Drosophila sturtevanti* and *Drosophila lehrmanae* (*saltans* group) *Bruna Roman*[1], Carolina Prediger[1,2], Amir Yassin[2], Lilian Madi-Ravazzi[1] 1) São Paulo State University (UNESP), Institute of Biosciences, Humanities and Exact Sciences, São José do Rio Preto, São Paulo, Brazil; 2) Laboratoire Evolution, Génomes, Comportement, Ecologie (EGCE), CNRS, IRD, Université Paris-Saclay, Gif-sur-Yvette, France

Wolbachia is an endosymbiotic genus of Alphaproteobacteria that infects a wide range of arthropods and nematodes. This endosymbiont is typically transmitted vertically, but in some cases, horizontal transmission has been reported. The horizontal transmission can occur in different ways in nature, such as parasitism, cannibalism and predation of infected individuals, hybrid introgression and shared ecological niches. Some species of *Drosophila saltans* group are infected by this endosymbiont, such as *Drosophila sturtevanti* (wStv), *D. prosaltans* (wPro) and *D. septentriosaltans* (wSpt). Here, we analyze the horizontal acquisition of *Wolbachia* in the host species *D. sturtevanti* and *D. lehrmanae*. We inferred a phylogenetic tree, in MrBayes, with 129 *Wolbachia* genomes (7 sequenced by us and 122 from database) and compared it with the phylogeny of the hosts. The single copy orthologs genes (17) were identified in OrthoFinder and used in the analysis. In general, the phylogeny generated showed that most strains were grouped into their defined supergroups according to the literature. However, six strains (namely, *w*Alb, *w*Agra, *w*Nik, *w*Adent, *w*Stv and *w*Stv-like), belonging to supergroup A, grouped together and distant from the other members of this supergroup. The formation of this last clade is interesting because the phylogeny of these strains demonstrates disagreement with the phylogeny of their hosts. Thus, this result suggests *w*Stv (host *D. sturtevanti*) and *w*Stv-like (host *D. lehrmanae*) were acquired by horizontal transmission, since *w*Pro and *w*Spt strains also infective of *saltans* group species were positioned phylogenetically

distant. This result suggests that this infection may have occurred in a common ancestor of *D. sturtevanti* and *D. lehrmanae*. The closest strain phylogenetically to *w*Stv and *w*Stv-like is *w*Adent, which infects a fungus-growing ant (*Apterostigma dentigerum*) belonging to the order Hymenoptera and family Formicidae. This host is widely distributed in Central and South America, co-occurring with *D. sturtevanti*, which provides some explanation for horizontal transmission. However, this question remains open, due to the lack of knowledge of the interaction of these host species. As new genomes are sequenced and added in this analysis, the route of this horizontal transmission may be clarified.

350V Sexual Selection is not a Driver of Female Sperm Storage Organ Length in *Drosophila* *Cameron Himes*, Tiffini Smith, Mollie Manier The George Washington University

In sexual selection, male trait evolution is often driven by female preference in a way that can generate exaggerated male phenotypes via a Fisherian runaway process. In this way, character transitions in the male trait are expected to follow female preference transitions. However, factors driving the evolution of female preference are less understood. Sperm in Drosophila are extremely long and are stored within the long, coiled female sperm storage organ, the seminal receptacle, or SR. When females mate with multiple males, long SRs favor long sperm, and short SRs favor short sperm. Thus, sperm length and SR length are a post-copulatory male trait-female preference system. Consistent with this, sperm length and SR length are coevolving across the Drosophila lineage, driven by a genetic correlation between the two traits. However, it is unknown what drives SR evolution. To test the hypothesis that SR length evolution is correlated with post-copulatory sexual selection, we obtained remating rates, SR length, sperm length, body size, and fecundity in 81 Drosophila species through direct experimentation and conducted a comprehensive literature search. We performed a comparative phylogenetic trait evolution test to determine if there is correlated evolution between remating rate and SR length in a phylogenetic context. There was no significant correlation between remating rate and SR length, suggesting that sexual selection is not driving the evolution of SR length on a macroevolutionary scale. The genetic correlation between SR and sperm lengths and their respective competitive phenotypes may be enough to drive sperm-SR coevolution without selection specifically acting on SR length.

351V Intermolecular interactions drive protein adaptive and co-adaptive evolution at both species and population levels *Junhui Peng*, Nicolas Svetec, Li Zhao Rockefeller University

Proteins are the building blocks for almost all the functions in cells. Understanding the molecular evolution of proteins and the forces that shape protein evolution is essential in understanding the basis of function and evolution. Previous studies have shown that adaptation frequently occurs at the protein surface, such as in genes involved in host-pathogen interactions. However, it remains unclear whether adaptive sites are distributed randomly or at regions associated with particular structural or functional characteristics across the genome, since many proteins lack structural or functional annotations. Here, we seek to tackle this question by combining large-scale bioinformatic prediction, structural analysis, phylogenetic inference, and population genomic analysis of Drosophila protein-coding genes. We found that protein sequence adaptation is more relevant to function-related rather than structure-related properties. Interestingly, intermolecular interactions contribute significantly to protein adaptation. We further showed that intermolecular interactions, such as physical interactions may play a role in the co-adaptation of fast-adaptive proteins. We found that strongly differentiated amino acids across geographic regions in protein-coding genes are mostly adaptive, which may contribute to the long-term adaptive evolution. This strongly indicates that a number of adaptive sites tend to be repeatedly mutated and selected in evolution, in the past, present, and maybe future. Our results highlight the important roles of intermolecular interactions and co-adaptation in the adaptive evolution of proteins both at the species and population levels.

352V Synthetic evolution of a *Drosophila* developmental network predicts trends in wild populations *Xueying Li*, Lautaro Gandara, Kerstin Richter, Justin Crocker EMBL

It remains unknown how developmental regulatory networks evolve, the predictability of their evolution, or to what degree laboratory evolution can be used to explore these processes. To address these questions, we have used experimental evolution to examine the *bicoid* (*bcd*) network in *Drosophila,* which is essential for anterior-posterior patterning in early embryos. This network can be synthetically perturbed by increasing the dosage of *bicoid*, which causes a posterior shift of the networks' regulatory outputs and a decrease in fitness. To directly monitor network evolution across populations with two extra copies of Bicoid, we performed unbiased genome-wide mutagenesis, followed by experimental evolution. We find that the evolved populations have increased fitness, canalization of gene expression, and normalized cuticles after ten generations. Using a multi-omics approach across the evolved populations, we find that there are increases in embryo length associated with maternal changes in metabolism and ovariole development. Consistent with our observation in laboratory evolution, we find that a wild population with larger embryos similarly rescues progeny with increased *bcd* expression. Together, our results necessitate a broader view of regulatory network evolution at the system level, and such knowledge learned from experimental evolution can help predict evolutionary trends in nature.

353V *fushi tarazu* and *fushi tarazu factor 1*, novel re-wiring in the *Tribolium castaneum* pair-rule gene network *Ximena Gutierrez Ramos*, Patricia L. Graham, Leslie Pick University of Maryland, College Park

Segmentation is a fixed feature of the insect body plan, yet surprisingly, the genetic network controlling the formation of segments has changed over the course of insect evolution. In *Drosophila*, pair-rule genes are responsible for allocating groups of cells to specific body regions that develop into separate, morphologically distinct body segments. Transcription factor (TF)-encoding genes regulate sets of downstream genes that include those encoding other TFs, signaling proteins, and products directly involved in morphogenesis. The evolutionary variation seen for pair-rule genes contrast with the conservation of expression of downstream target genes. How does the regulatory network re-wire to maintain downstream gene expression while upstream regulators are lost, gained, or changed in function? We are exploring this question by examining the expression, function, and interactions of the pair-rule gene *fushi tarazu (ftz)* in the flour beetle, *Tribolium castaneum (Tc)*. *Tc*-FTZ contains an LXXLL motif that is necessary for the functional interaction of FTZ and its obligatory partner FTZ-F1 in *Drosophila*. Despite this, a large genomic deletion (Stuart *et al.,* 1991) and RNAi experiments (Choe *et al.,* 2006 and data not shown) indicate that *Tc-ftz* plays no role in segment formation in *Tribolium*. In contrast, FTZ-F1 is expressed in pair-rule stripes in *Tribolium* and RNAi knockdown resulted in pair-rule defects (Heffer *et al.,* 2013). To definitively determine whether *Tc-ftz* plays a role in segmentation, we are using CRISPR/Cas9 to generate a genomic deletion of this gene. Further, to understand how FTZ-F1 regulates pair-rule patterning in *Tribolium* without the input of FTZ, we are examining cis-regulatory elements of the conserved target gene *engrailed* (*en*). We are testing approaches to using CRISPR-based and piggybac-based enhancer reporter genes to identify *en* regulatory elements and assess their dependency on *Tc*-FTZ-F1, using dsRNA to knockdown *Tc-ftz-f1* expression. Other TFs binding to identified cis-regulatory elements will be identified and characterized. Simultaneously, we have generated a FLAG-tagged version of *Tc*-FTZ-F1 to identify other genomic targets, determine whether or not *Tc*-FTZ plays a role in their regulation and to identify the DNA binding partners using co-immunoprecipitation. This will allow us to understand how a rearrangement of upstream regulators occurs to maintain a conserved downstream gene expression pattern, and provide information on the transcriptional regulation of the downstream genes within the *Tribolium* pair-rule gene network.

354V Probing evolution by *Hox* locus replacement *ANKUSH AURADKAR*[1,2], Emily A. Bulger[3], Sushil Devkota[1], William McGinnis[1], Ethan Bier[1,2] 1) Section of Cell and Developmental Biology, University of California San Diego, La Jolla, CA 92093, USA; 2) Tata Institute for Genetics and Society-UCSD, La Jolla, CA 92093-0335, USA; 3) Developmental and Stem Cell Biology Graduate Program, University of California San Francisco, and Gladstone Institutes, San Francisco, CA, USA

Hox genes determine positional codes along the head-to-tail axis. Here, we replaced the entire *Drosophila melanogaster proboscipedia (pb) Hox* locus, which controls the development of the proboscis and maxillary palps, with that from *Drosophila mimica,* a related species with highly modified mouthparts. The *D. mimica* replacement rescues most aspects of adult proboscis morphology; however, the shape and orientation of maxillary palps were modified, resembling *D. mimica* and closely related species. Expressing the *D. mimica* Pb protein in the *D. melanogaster* pattern fully rescued *D. melanogaster* morphology. In contrast, expression of the *pb* locus directed by *D. mimica pb* cis-regulatory sequences was reduced from that of *D. melanogaster pb* in cells that produce altered maxillary structures, potentially altering the balance between *pb* and a competing *Hox* gene *Deformed* (*Dfd*). This hypothesis is consistent with the observation that over-expression of *Dfd* in *D. melanogaster* results in altered maxillary palp orientation similar to that in *D. mimica* replacement. These findings suggest that *pb* regulatory sequences evolved in related species to alter mouthpart morphology.

Auradkar, A., Bulger, E. A., Devkota, S., McGinnis, W. & Bier, E. Dissecting the evolutionary role of the *Hox* gene *proboscipedia* in *Drosophila* mouthpart diversification by full locus replacement. *Sci. Adv.* **7**, 1003(2021).

355V Genetic architecture of male-female coevolution in Drosophila melanogaster Cameron Himes, Galvin Jake, *Mollie Manier* George Washington University

In sexual selection, male trait evolution is often driven by female preference in a way that can generate exaggerated male phenotypes via a Fisherian runaway process. This male-female co-evolution requires a genetic correlation between the male trait and female preference, but the genetic architecture behind this correlation is typically unknown. In Drosophila, sperm are extremely long and are stored within the long, coiled female sperm storage organ, the seminal receptacle, or SR. SR length is a mechanism of cryptic female choice for sperm length, such that when females mate with multiple males, long SRs favor long sperm, and short SRs favor short sperm. Thus, sperm length and SR length are a post-copulatory male trait-female preference system. Consistent with this, sperm length and SR length are coevolving across the Drosophila lineage, driven by a genetic correlation between the two traits. Here, we have used the Drosophila Synthetic Population Resource (DSPR) to characterize the genetic architecture underlying sperm-SR coevolution and to identify candidate genes underlying each trait. We found that sperm and SR length are each associated with a single QTL peak that are closely linked on chromosome 3R, leading us to conclude that the genetic mechanism of male-female

co-evolution in this case is linkage rather than pleiotropy. Within the sperm QTL is the gene scotti (soti), which encodes a testis-specific protein that localizes to late-stage spermatids and regulates the onset of individualization. We examined soti mutants and found that spermatid cysts are 350 microns shorter than wild type controls. Within the SR QTL are two developmental regulators of epithelial cell migration, heartless (htl) and stripe (sr). Both of these genes are expressed in the female reproductive tract and enriched in SR tissue, which is epithelial in origin. Ongoing experiments are further characterizing the roles of htl and sr in SR development.

356V Genetic basis of variation in high sugar-induced diabetes-associated traits and development delay in *Drosophila* *Xuan Zhuang*[1], Fabio Morgante[2], Abaranjitha Muniyasamy[1], Michael Ludwig [3], Soo Young Park [3], Yang Li[3], Matthew Stephens[3], Graeme Bell [3], Martin Kreitman[3] 1) Department of Biological Sciences, University of Arkansas, Fayetteville, AR; 2) Clemson Center for Human Genetics, Clement University, Clemson, SC; 3) Department of Ecology and Evolution, University of Chicago, Chicago, IL

Drosophila is a well-established model for investigating complex traits and many human diseases. It provides powerful tools for dissecting the contributions of both genes and environment on development and metabolism. We developed a *Drosophila* model to study high sugar diet (HSD) induced Type 2 diabetes (T2D) associated traits in large populations with different genetic backgrounds. We examined HSD induced phenotypes in larvae and in adults across a subset of *Drosophila* Genetic Reference Panel (DGRP). Flies on HSD display an increase in whole body glucose and glycogen level, a decrease in developmental rate, survivorship, body weight, and longevity, compared with flies under low sugar diet (LSD). The examined DGRP lines display a continuous and wide range of these phenotypes and large broad-sense heritability, suggesting great potential for quantitative trait loci (QTL) mapping. In the meanwhile, we developed a unique experimental system for genetic mapping named Drosophila Recombinant Populations (DRPs), which are consisted of 16 outbred advanced intercross populations (AIPs), each founded with 8 inbred DGRP lines. The DRPs provide about 70,000 genotypically distinct flies that allow us to apply the HSD induced T2D model to each individual to investigate the genetic architecture of these complex traits. We used one of the HSD induced traits, namely developmental delay, to perform a bulk segregant mapping analysis of extreme phenotypes. We developed a computational pipeline with a Hidden Markov model (HMM) to impute the whole genome of each fly based on their low-coverage sequenced genomes and the available high-coverage founder genomes. Genome-wide association studies (GWAS) identified 76, 2009, and 373 polymorphisms at $p<10^{-5}$ for the analysis of HSD, LSD, and G x E (gene by diet) respectively. Gene ontology (GO) analysis indicates predominant enrichment for genes involved in some hormones and ketones cellular metabolic processes among HSD candidates, and reproduction and mediator complex among LSD candidates. RNAi validation studies of the top candidates (Cyp9b2 and CG15088 from GxE analysis, wun and Ten-m from HSD analysis) indicated their moderate but still significant effects in increasing the development rate on HSD. This study provides a broad survey of diabetes associated traits in HSD induced T2D flies, and further dissects the genetic architecture of development time on diets of different sugar concentrations, and interaction of gene and diet.

357V Effective label of XL/XR and Neo-X chromosomes of *Drosophila miranda* using oligopaints probes *Henry Bonilla*[1], Isabela Pimentel de Almeida[1], Mara Lisboa Santana Pinheiros[1], Maria Vibranovski[1,2] 1) Bioscience Institute, University of São Paulo; 2) New college for Interdisciplinary Arts and Sciences, Arizona State University

Experiments of fluorescent in-situ hybridization (FISH) are a valuable resource for investigating chromosome evolution, chromosome behavior in individual cells and, ultimately, for assessing the quality of a sequenced genome. *Drosophila miranda* is a model species to study the evolution of sex chromosomes due to their X chromosomes of different ages. The outcome of independent autosome-sex chromosome fusions produced the so-called XR and Neo-X that emerged about 15 MYA and 1.5 MYA ago, respectively.
Oligopaint represented a new generation of probes (Beliveau et al 2012) that are custom-synthesized oligonucleotides, short (≈26–46 bases), versatile and very specific for targeting single-copy chromosome regions that can be extended to any organism whose genome has been sequenced. Here, we developed oligopaints to label the entire set of X (XL/XR and Neo-X) and the fourth autosomal chromosomes. We used OligoMiner pipeline (Beliveau et al., 2018) and the latest *D. miranda* genome sequence (Manhajan et al., 2018). The specificity of oligopaints was assessed by FISH experiments on mitotic chromosomes. Shortly, we designed oligopaints under the stringent condition defined in *OligoMiner* in addition to Kmer and secondary structure filters to guarantee specificity. A Python script was developed to diminish the number of oligos but maintain an appropriate distribution along entire target regions. Finally, flanking primer regions were added to produce oligos from a custom complex oligo-pool library.
We obtained 25553 oligos covering the entire Neo-X chromosome with a density of 1.01 oligos/Kb. The XL/XR chromosome has been divided in four regions according to their different evolutionary history and the genome assembly. By separating the XL arm in pericentromeric and distal regions (XL-1 and XL-2), we obtained 11180 oligos (0.79 oligos/kb) and 27282 (1.08 oligos/kb), respectively. The XR arm was split in XR-A and XR-D. XR-A comes from the ancient X chromosome and it's located in the pericentromeric region whereas XR-D in the distal portion of the XR arm. We obtained 12717 oligos (1.02) and 25382 oligos (0.99) in the XR-A and XR-D respectively. Only 10336 oligos (0.32 oligos/kb) were designed for the fourth chromosome.

FISH experiments on mitotic chromosomes revealed that all of our oligos are target region specific. A complete label on Neo-X and on the fourth chromosome was observed, despite the low oligo density of the latter. This finding is valuable, as there are not many studies on the minimum density of oligos necessary for visible marking along entire chromosomes. Finally, the XL/XR chromosome showed almost complete labeling. There is only a small region between XL-1 and XL-2 that has not been labeled because it could not be assembled into the genome. Our results represent the first oligopants designed for *D. miranda* which will allow further research on sex chromosomal behavior in different cell types.

358C Signals governing pupal development of ovarian Follicle Stem Cells and Niche Cells *Rachel Misner*, Helen Kogan, Daniel Kalderon Columbia University, Department of Biological Sciences, New York, NY

The follicle stem cell (FSC) niche in the adult *Drosophila* ovary balances the actions of stem cell self-renewal and output through the guidance of graded extracellular signals. Anterior sources (cap cells, escort cells [ECs]) produce Hh and Wnt pathway ligands, and posterior sources (polar follicle cells [FCs]) produce JAK-STAT pathway ligands. These opposing gradients intersect over the FSC domain, and regulate FSC division and differentiation into quiescent ECs (anterior) or proliferative FCs (posterior) across FSC positions. We have previously shown through combined lineage and morphological studies that adult ECs, FSCs, and FCs derive from intermingled cell (IC) precursors present at the start of pupation, and acquire their adult function based on their final anteroposterior (AP) location, rather than a series of cell fate decisions. Our studies also revealed how the first FCs and egg chamber are formed. Precursors accumulate posterior to germline and ICs of the developing germarium. These Extra-Germarial Crown (EGC) and Basal Stalk (BS) cells are then invaded by the most posterior germline cyst without any attached ICs, and surround this cyst in an epithelial monolayer. Here we investigate how signaling pathways influence somatic cell AP locations and adult fates during pupal development. Wnt signaling initiates early from the anterior. MARCM lineage studies showed that cells with elevated Wnt pathway activity are biased towards more anterior locations, while reduced Wnt signaling favored more posterior locations. Detectable JAK-STAT signal was seen only after budding of the first pupal egg chamber, when polar FCs are specified. Loss of JAK-STAT signaling favored more anterior locations. Elevated JAK-STAT signaling increased precursor output, suggesting faster division. Hh signaling has previously been demonstrated to begin before pupal development and to be important for generating adult somatic cells of the ovary. Preliminary results suggest that Hh signaling during pupal stages is required for ovarian somatic cell survival and proliferation, with effects on cell location still to be determined. We are particularly interested in exploring which signals affect formation of the first FCs because that process occurs in a different morphological setting to the conversion of adult FSCs to FCs.

359A Diapause extends female germline stem cell longevity in *Drosophila* *Sreesankar Easwaran*, Denise Montell University of California - Santa Barbara

A consequence of aging in many species including humans is reduced female fertility. Intriguingly, some species can preserve fertility longer under specific environmental conditions. For example, at low temperature and short day-length, *Drosophila melanogaster* can enter a dormant state called adult reproductive diapause. During this cold-induced quiescence, as in other stressful conditions, ovarian development arrests at the yolk uptake checkpoint. However, mechanisms underlying fertility preservation and post-diapause recovery are largely unknown. Here, we report the effects of cold-induced arrest and recovery on Drosophila ovarian development. We found that diapause affects all stages including germline stem cells (GSCs) and is distinct from other stress responses in causing more complete arrest yet preserving the potential for recovery. Further, GSCs incur DNA damage, activate p53 and the Chk2 checkpoint, and divide less during dormancy. Niche signaling is reduced yet germline precursor cells do not differentiate. Thus, GSCs adopt an atypical, suspended state connected to cystoblast daughters. Post-diapause recovery of niche signaling, resumption of GSC division, and dedifferentiation contribute to restoring GSCs. Mimicking one feature of the quiescent state, reduced juvenile hormone production, enhanced GSC longevity in non-diapausing flies. Thus, studies of adult reproductive diapause in Drosophila can provide approaches to GSC longevity enhancement.

360B The impact of cell cycle and DNA damage response on germline stem cell survival in the *Drosophila* testis *Jasmine Grey*, Salman Hasan, Erika Matunis Johns Hopkins University School of Medicine, Baltimore, MD

Stem cells are integral to the development and maintenance of a tissue. Adult stem cells have the unique ability to self-renew and produce differentiated daughter cells and have been shown to survive radiation-induced DNA damage in many tissues. However, the exact pathways these cells use to repair DNA damage in an intact tissue are not very well understood. Here we use the *Drosophila* testis stem cell niche to understand the processes of DNA damage response in an intact niche. Germline stem cells (GSCs) give rise to sperm in the *Drosophila* testis and have been shown to resist high (75 Gy) irradiation (IR), while their differentiating progeny, spermatogonia die. The radio-resistance of the GSCs suggests that they efficiently and successfully use the DNA double-strand break (DSB) repair pathways, non-homologous end joining (NHEJ) and homologous recombination (HR). Our goal is to understand the specific mechanisms GSCs use to resist IR induced damage and maintain tissue homeostasis. Preliminary data suggest that the response of GSCs to IR in flies null for NHEJ is phenotypically indistinguishable from wild type, but flies null for HR are more sensitive to IR. We hypothesize

HR is required for damage-induced regeneration and that the ability of the GSCs to survive irradiation depends on its cell cycle phase at the time of irradiation, which dictates the DSB repair pathway used. To test this, we are using *Drosophila*-specific fluorescent ubiquitination-based cell cycle indicator (fly-FUCCI) system to visibly mark the stages of the cell cycle in live and fixed imaging, providing an in-depth analysis into the survival mechanisms carried out by IR-damaged GSCs. A deeper understanding of stem cell resistance to IR will add greatly to the *Drosophila* testis field and may have implications in other radio-resistant stem cells, such as cancer stem cells.

361C Tnpo-SR maintains ovarian cyst connectivity and is required for GSC fusome dynamics morphogenesis in *Drosophila* ovarian germline stem cells *Anna Williams*, Elizabeth Ables East Carolina University

The maintenance of species is dependent on parental genetic information being passed onto the next generation. Germ cells are crucial for this transfer and therefore drive evolution. Evolutionarily conserved across phyla, germ cells in many systems are supported by a stem cell population (GSCs). In *Drosophila*, these stem cells divide via incomplete cytokinesis to form interconnected daughter cells that will mitotically divide and differentiate to form oocytes and nurse cells, which supply the oocyte with maternal factors necessary for embryogenesis. Connection of daughter cells is facilitated by the fusome, an endoplasmic reticulum-like structure with cytoskeletal and membranous components. The fusome is remodeled after every division and becomes increasingly branched as the cysts continue to divide. Tnpo-SR, an ecdysone-responsive gene, was identified in a genetic screen for its putative role in stem cell self-renewal. Tnpo-SR shares amino acid similarity to mammalian β-importin proteins; however, its specific roles in gamete production has not been characterized. Using genetic mosaic mutants alongside germline-specific RNAi, we found that Tnpo-SR is necessary in GSCs for their self-renewal. Depletion of *Tnpo-SR* in dividing germ cells generates egg chambers with cells varying from the typical 16 suggesting abnormal cyst development and cyst fragmentation. Here, we investigated the cytoskeletal dynamics of Tnpo-SR mutant GSCs and cystoblasts in order to further characterize the role of Tnpo-SR in GSC self-renewal. We find that GSC fusome density is decreased when *Tnpo-SR* is depleted, suggesting its role in GSC fusome morphogenesis. The alteration of cyst formation also impacts oocyte selection. This research identifies a novel role of the karyopherin Tnpo-SR on not only GSC formation and maintenance but also the formation of a healthy egg needed to drive the maintenance of species.

362A Effects of nuclear lamina aging on oogenesis *William Zaremba*, Pamela Geyer University of Iowa

Tissue homeostasis depends on replacement of injured and defective cells by proliferation of resident adult stem cells. Homeostasis declines with age, largely due to decreased stem cell function and/or number. The Drosophila ovary provides an outstanding model for investigating age effects on tissue homeostasis, as oogenesis depends upon a well-defined germline stem cell (GSC) population that sustains oocyte production for many weeks. Age-dependent declines of oocyte production are associated with decreased mitosis, not GSC loss. Strikingly, we discovered that the asymmetric mitoses of GSCs are specialized, wherein the nuclear envelope and nuclear lamina remain throughout mitosis. During these specialized mitoses, spindles are nucleated from centrosomes embedded in the retained NL. The mode of GSC mitosis raises questions about how the mitotic NL contributes to homeostasis. To this end, we have studied age effects on accumulation of mitotic NL components, finding that lamin-B and emerin levels decline with age. These observations support predictions that deterioration of mitotic NL integrity reduces mitotic division and oocyte production. Further support for this prediction comes from our studies of the role of Checkpoint kinase 2, a transducer kinase that becomes activated upon NL defects and elevated DNA damage in germ cells. Notably, loss of Chk2 prolongs oocyte production, delaying loss of egg laying by 15 days, suggesting that activation of Chk2 contributes to reduced fecundity of older females. Taken together, our studies provide new insights into age-dependent contributions of the NL to homeostasis of the ovary.

363B Function of Bazooka in dedifferentiation of the male germline stem cells *Muhammed Burak Bener*, Mayu Inaba University of Connecticut Health, Farmington, CT

Drosophila male germline stem cells (GSCs) divide asymmetrically to generate a self-renewing stem cell and a differentiating cell. The outcome of asymmetric division is compromised when cells that once initiated differentiation program revert back to stem cell state (i.e., dedifferentiation). Dedifferentiation has been proven to be an essential mechanism for the maintenance of the GSC pool. However, compromised asymmetric outcome may result in the clonal expansion/depletion of stem cells, leading to various pathological conditions. Therefore, the process of dedifferentiation must be carefully regulated. In this study, we characterized the process and outcome of dedifferentiation to understand the underlying mechanisms. By experimentally inducing a GSC-loss and recovery cycle in the testis, we found that the dedifferentiated GSCs reestablish the patch-like structure of a polarity protein, Bazooka (Baz, *Drosophila* homolog of mammalian Par-3), at the hub-GSC interface quickly within 1 to 3 days after induced GSC loss. Moreover, we found that differentiating spermatogonia start expressing Baz prior to migration to the niche, which suggests a role of Baz in the regulation of the dedifferentiation process. Baz has been shown to be required for stem cell-specific polarity checkpoint, centrosome orientation checkpoint (COC). Consistent with the quick recovery of Baz-patch, we observed the presence of intact centrosome orientation checkpoint, correct spindle orientation, and intact mitotic rates in dedifferentiated

GSCs. Moreover, our live observation demonstrates that dedifferentiated GSC immediately divides after positioned back to the niche. Taken together, our results demonstrate that dedifferentiated GSCs quickly regain the ability to undergo asymmetric cell division and Baz plays an essential role in this process.

364C Investigating the Regulation of Germline Stem Cell Cytokinesis by Somatic Stem Cells *Carlos Billini*, Kari Lenhart Drexel University

Most adult tissues have multiple specialized stem cell populations residing within a single microenvironment, or niche, that repopulate and regenerate the tissue over time. While much is known about how the niche controls a single stem cell line, not much is known regarding the interaction and coordination between the different stem cell types contained by a single niche. The *Drosophila* testis niche contains two populations of stem cells: Cyst Stem Cells (CySC) and Germline Stem Cells (GSC) that must be precisely coordinated for proper tissue function. It is known that two CySC daughter cells envelope a single GSC daughter cell, insulating the GSC daughter from niche signals. Our lab has identified that somatic control of GSC cytokinesis helps coordinate daughter cell release from the niche, resulting in this 2:1 ratio. Further, we determined that the 2:1 ratio is required for complete and proper GSC daughter cytokinesis. Inhibition of CySC encystment leads to failure of abscission, or membrane severing, between GSC-daughter pairs and results in failure of release of a differentiating germ cell from the niche. Therefore, we hypothesize that a signal from CySCs is necessary to trigger proper abscission of the GSCs. We have additionally found that Wnt pathway activation within CySCs is required for successful completion of GSC abscission. Surprisingly, we find that somatic Wnt pathway activation does not directly regulate GSC abscission. Instead, we have discovered that Wnt activity in CySCs is necessary to dampen GSC cycling rate. Inhibition of the Wnt pathway in somatic stem cells results in a significant increase in GSC cycling rate and a consequent failure of these cells to successfully complete cytokinesis prior to mitotic entry. Further understanding how somatic stem cells regulate the cycling rate of adjacent GSCs will elucidate the collaborative mechanisms in place within the niche to ensure tissue homeostasis.

365A Investigating re-initiation of stem cell cytokinesis during tumor proliferation *Beth Kern*, Kari Lenhart Drexel University

Adult stem cells become depleted throughout the lifetime of an organism, necessitating regenerative mechanisms to maintain the stem cell niche. Interestingly, signals for plastic regeneration mechanisms are often shared with those initiating tumorigenesis. A common feature of both plasticity and tumorigenesis is a reversion of differentiating cells to a stem-like state. The *Drosophila* male testis provides an invaluable model to study the process whereby differentiating cells transform into overproliferative tumor cells due to its distinct location and genetic tractability. Within the testis, the stem cell niche maintains both germline stem cells (GSCs) and somatic cyst stem cells (CySCs). GSCs divide asymmetrically to produce a differentiating daughter, which must become encapsulated, or encysted, by two CySC daughters. Our lab has developed a model whereby the coordinated release of this 2:1 soma-germline grouping is achieved via a modified cytokinesis program that pauses completion of GSC cytokinesis until somatic encystment of the germline is achieved. Whether this modified cytokinesis program becomes re-engaged during tumorigenesis and to what degree tumorous germ cells reinstate stem cell specific processes has yet to be explored. By genetically inducing tumorigenesis through ectopic expression of niche signals, we have visualized actin misregulation at the intercellular bridge and subsequent tumor fractionation by single cell abscission. We now aim to directly examine if tumor progression requires re-initiation of the stem cell-specific cytokinesis program. Examination of *in vivo* tumor proliferation will provide critical insights into the cell biology underlying tumorigenesis and may be useful in elucidating novel therapeutics for treatment of carcinoma.

366B Programmed changes of interaction of Stat92E homologous loci regulate transcription during the stem cell differentiation Mayu Inaba, *Matthew Antel* UConn Health, Farmington, CT

Correlation between strength of pairing of homologous chromosomes and gene expression status has been demonstrated in genome-wide, implicating the functional impact of homolog pairing on local transcriptional activity. *Drosophila* male germline stem cells (GSCs) constantly divide asymmetrically to produce one GSC and the other daughter as a differentiating gonialblast (GB). GB then enters differentiation program in which stem cell specific genes are quickly downregulated. Here we demonstrate that a change of local pairing status of homologous Stat92E loci is required for downregulation of Stat92E gene during the differentiation. Using Oligopaint fluorescent in situ hybridization (FISH) technique, we found that the interaction between homologous loci of Stat92E is always tight in GSCs and immediately loosened in GBs. When one of the STAT92E loci was absent or relocated on other chromosome, Stat92E did not pair and failed to downregulate. Same defect was observed upon knocking down of pairing factors, suggesting that the pairing is likely required for switching transcriptional status. Moreover, Stat92E enhancer element but not cis-transcription is required for the change of pairing status, indicating that pairing change is not a consequence of transcriptional changes. GSCs are known to inherit pre-existing histones (H3 and H4), while newly synthesized histones are distributed in GBs. When this histone inheritance was compromised, the change of Stat92E pairing did not occur, suggesting that the pairing change is an intrinsically programmed process during the asymmetric stem cell division. Taken

together, we propose a possibility that the change of local pairing status may be a common process to promote rewriting gene activity status during cell-differentiation.

367C Examining the Role of Adipokines in Regulating Oogenesis *Chad Simmons*, Alissa Armstrong University of South Carolina

Obesity and malnutrition can influence nutrient signaling pathways that relay nutrient status throughout the body, yet the molecular components that allow communication about dietary input between different tissues is not completely understood. *Drosophila melanogaster* ovarian function is sensitive to changes in diet and serves as an ideal model to decipher how nutrient sensing by peripheral tissues influence the ovary. Previous studies demonstrate that *Drosophila* adipose tissue uses nutrient sensing pathways, like insulin/insulin-like growth factor and Target of Rapamycin signaling to modulate ovarian germline stem cells and their progeny (Armstrong et al., 2014; Armstrong and Drummond-Barbosa, 2018). Multiple adipokines, factors secreted from the adipose tissue, are required for tissue growth during larval development. The range of adipocyte-derived factors that contribute to adipocyte-to-ovary communication is not well understood. We propose that the adipokines with roles in larval growth, eiger (egr), unpaired2 (upd2), stunted (sun), growth blocking peptides (Gbp1, Gbp2, Gbp3) and CCH-amides (ccha2) also function to promote adipose tissue nutrient sensing control of oogenesis. We find that all are expressed in the adult female fat body. Using RT-PCR, we are determining if their expression is modulated by protein-poor and obesogenic diets. To determine their functional role, we use the *Gal4/UAS* gene expression system for RNAi-mediated knockdown of each adipokine specifically in adult adipocytes and measure effects on oogenesis using whole mount immunofluorescence and confocal microscopy. Thus far, we have observed that adipocyte knockdown of *upd2* and *egr* leads to increased adipocyte size but does not affect germline stem cell number in the ovary. We are currently investigating the effects of adipokine knockdown on other aspects of oogenesis, including germline stem cell maintenance, germline cyst survival, progression through vitellogenesis and ovulation. Understanding how these adipokines communicate to the ovary will allow us to begin uncovering the cellular and molecular mechanisms that underlie signaling between adipose tissue and the germline stem cells.

368A Assessing the interactions between *W. pipientis* genotype and titer on the *bag of marbles* partial loss of function mutant (hypomorph) in *Drosophila melanogaster* *Catherine Kagemann*, Charles Aquadro 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. However, there are other known interactions between *W. pipientis* and its host. For example, *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 for the production of 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 have yet to determine the functional mechanisms that are behind this interaction. Therefore, we aim to elucidate 1) whether variation in *W. pipientis* variant genotype and titer influence the rescue of the mutant *bam* phenotype at different ages in adult females and 2) whether *W. pipientis* variants cause differential rescue of the *bam* hypomorph phenotype at the transcriptional level as the host fly ages. Results show that rescue of the mutant *bam* phenotype does in fact depend on the genotype of *W. pipientis* as the flies age and the magnitude of rescue is dependent on the age of the female fly. Relative quantification of *W. pipientis* titer via qPCR shows that titer increases in all *W. pipientis* genotypes at the peak rescue of the *bam* hypomorph phenotype. 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. Additionally, pairwise comparisons between *W. pipientis* genotypes showed differential expression of one of *bam*'s genetic interactors, *zpg*, and several other GSC genes. RNAi will be used to determine whether any of these candidate genes identified from our RNA-seq analysis are responsible for aiding in the rescue of the *bam* hypomorph phenotype at the transcriptional level.

369B Lineage decisions and competency in early *Drosophila melanogaster* neurogenesis *Fiona Kerlin*, Robert Zinzen Max Delbrück Center for Molecular Medicine in the Helmholtz Association Berlin-Mitte (BIMSB)

During embryogenesis in Drosophila melanogaster, a tightly controlled gene regulatory network generates a specific number of neurogenic stem cells, also called neuroblasts, in distinct positions over the course of early development. This process of neurogenesis generates a total of ~30 neuroblasts per segment in a spatially and temporally controlled manner, where neuroblasts are successively selected and delaminate from an epithelial sheet of cells along 3 dorsoventral columns and several anteroposterior rows. Once born, neuroblasts divide asymmetrically several times to self-renew and produce ganglion mother cells, neurons, and glia. This series of events is characterized by a temporally and spatially distinct gene expression, so that each neuroblast has a unique pattern of gene expression that determines its developmental trajectory. Importantly, the spatial and temporal origin of the neuroblast is thought to determine its

ensuing lineage and cell fate.
After decades of research the neurogenic system in the early Drosophila embryo is quite well understood phenomenologically: Not only are we aware of many gene expression combinations that are distinct for individual neuroblasts, but fluorescent labeling experiments of individual neuroblasts have yielded maps of their developmental trajectories. However, our understanding of the molecular mechanisms that restrict, drive and distinguish neuroblast fate decisions is extremely limited.
New studies in my lab using single-cell sequencing are for the first time able to assess the genome-wide gene expression complements of individual neuroblast identities with the aim to determine the expression of factors that delineate these identities.
Mapping cell fate decisions over the course of embryonic neurogenesis of *Drosophila* will allow for a more comprehensive understanding of lineage decisions that give rise to the emerging neurogenic cell identities in the early nervous system. My aim is to reveal the exact lineages that give rise to neurons and glia with spatial resolution in vivo. Specifically, the building of a lineage tree for neuroblasts will allow to infer a relatedness of the cells to each other. So far only the common ancestry of cells could be shown, but not when and in which succession those cells separated from each other.
I aim to map lineage decisions and couple the description of lineages with knowledge of when and where molecular markers are expressed.
The lineage of neuroblasts will be recorded with the intMEMOIR method, that relies on a genomic editing process via an integrase and makes cells express the resulting barcode. Image acquisition of the barcode transcript with RNA in situ hybridization will be followed by lineage tree modelling. This should lead to the identification of new marker genes and foster a more detailed understanding about the underlying molecular mechanisms that drive early neurogenic trajectories.

370C Neural Circuits Involved in Nutrient-Dependent Neuroblast Reactivation *Susan Doyle*, Cami Kellinui, Xin Yuan, Sarah Siegrist University of Virginia, Charlottesville, VA

The precise control of cell growth and division is critical for proper tissue development, integrity, and function. Growth control involves the integration of intrinsic cellular programs with extrinsic cues, including growth factors, hormones, and the availability of nutrients. At the end of Drosophila embryonic development, proliferating neural stem cells, known as neuroblasts, enter into a period of mitotic dormancy called quiescence. Neuroblast exit from quiescence and cell cycle resumption is tightly coupled to nutrient availability; as larvae hatch and begin feeding, amino acids are sensed, resulting in release of Drosophila insulin-like proteins (Dilps) and subsequent activation of the PI3K growth pathway in neuroblasts. Both Dilp2 produced by insulin producing cells (IPCs) in the brain and Dilp6 from glial cells have been shown to mediate the PI3K activation in neuroblasts that results in exit from quiescence. The fat body, a key nutrient sensing tissue, has likewise been shown to be important for both sensing amino acids and producing systemic factors that result in Dilp secretion and neuroblast reactivation in the larval CNS. Here, using animals in which the fat body has been ablated and cultured brain explants from freshly hatched larvae, we demonstrate that the fat body is not required per se for nutrient-dependent reactivation of neuroblasts from quiescence. This suggests that other cell types and tissues capable of amino acid sensing, local brain production of Dilps, and/or uptake of Dilps from IPCs may be capable of driving nutrient-dependent neuroblast reactivation. We further explore this hypothesis using the trans-Tango and DenMark systems to trace pre- and post-synaptic contacts of Dilp2 and Dilp6 producing neurosecretory neurons as well as neurons in the brain expressing the insulin binding protein ImpL2. Using the GAL4/UAS system to express the proapoptotic gene grim and the potassium channel kir2.1 for synaptic silencing, we test the role of specific sets of neuropeptide and neurotransmitter-producing neurons in reactivation of neuroblasts in response to nutrients. Our results shed light on the programs that govern neural stem cell proliferation decisions in response to dietary nutrient availability.

371A A Screen for Amino Acid Transporters Involved in Nutrient-Dependent Reactivation of Quiescent Neuroblasts *Erik Miao*, Jonathan Day, Susan Doyle, Bharath Sunchu, Sarah Siegrist University of Virginia

In the developing *Drosophila* nervous system, neural stem cells, called neuroblasts, undergo a period of quiescence at the end of embryogenesis. Neuroblasts exit from quiescence after larvae hatch and begin feeding. Dietary amino acids are required for neuroblast reactivation, however little is known regarding the exact nature of amino acids required and the cell and tissue types involved in sensing them. Insulin signaling is a key regulator of nutrient-dependent growth and metabolism in neuroblasts, and Drosophila insulin-like peptides (Dilps), produced by the insulin-producing cells (IPCs) of the brain, activate PI3-kinase signaling in neuroblasts. Recently, two L-type amino acid transporters expressed in the IPC's have been shown to be required for release of Dilp2 in response to the amino acid leucine. Therefore, we hypothesized that amino acids directly sensed by the IPCs may play an important role in nutrient-dependent reactivation of neuroblasts from developmental quiescence.

To test this hypothesis, we used the Gal4/UAS binary expression system to disrupt transmembrane amino acid transporters in the IPCs. UAS-RNAi lines for the 26 genes in the solute carrier family that encode AA transporters were

expressed under the control of an IPC-specific GAL4 driver (dilp2-GAL4), in combination with a pcnaGFP transgene to assess cell proliferation. Freshly hatched larvae were raised on a standard Bloomington diet, and after 24 hours of feeding, live larvae were removed from food, rinsed and mounted on a glass slide with a coverslip to immobilize them. Brains were imaged in whole mount through the cuticle using epifluorescence, and reactivation from quiescence scored based upon GFP expression.

After screening through 21 of the proposed RNAi lines, no significant reduction of reactivation was found with comparison to the control line (OregonR). This suggests that redundancy exists in nutrient uptake pathways, such that knocking out one AA transporter is not enough to show a significant reduction in reactivation. In the future, we plan to expand our amino acid transporter screen by using Gal4 drivers to express AA transporter RNAi lines in the fat body, glia, and neuroblasts themselves. The results of these experiments may give us valuable information on the tissues and nutrients required to reactivate neural stem cells from quiescence, and in addition may lend insight into improving human stem cell regeneration.

372B Activin signaling controls ISC proliferation and cell fate to maintain adult gut homeostasis *Christian Christensen*, Julien Colombani, Ditte Andersen University of Copenhagen, Department of Biology

The intestinal tract is the most proliferative organ in the body, and coordination of intestinal stem cell (ISC) activity and cell fate is dynamically tuned to ensure epithelial integrity at homeostasis and in response to injury. To identify niche-derived signals controlling adult gut homeostasis, we recently carried out a large-scale functional genetic screen using RNAis targeting secreted peptides expressed in the ISC niche. Our screen identified several of the evolutionarily conserved TGF-beta/activin ligands suggesting an important function of activin signaling in controlling gut homeostasis. Consistent with this, we found that niche-derived activin-beta signals through the type I activin receptor, baboon, in the stem- and progenitor cells to promote regenerative growth in response to an oral *Ecc15* infection. Additionally, we find that activin signaling in intestinal stem- and progenitor cells participate in coordinating proliferation and cell fate decisions during homeostasis to restrict gut epithelial turnover rates and ensure gut integrity. Activin signaling was recently reported to regulate salivary gland stem/progenitor cell function in mice, suggesting a conserved role of activins in controlling adult stem cell activity and tissue homeostasis.

373C Functional analysis of Escargot and STAT targets in intestinal stem cells of the *Drosophila melanogaster* posterior midgut Armen Khanbabaei, Aaron Lemus, Cynthia Petrossian, Donnie Ca, Courtney Frazier, Ithan Cano, Marziiah Hossine, *Mariano Loza-Coll* California State University, Northridge, Los Angeles, CA

Many organs contain adult stem cells (ASCs) that replace cells lost to damage, disease or normal turnover. Like their embryonic counterparts, 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. Previous work has led to the identification of so-called master regulators (MR) genes in ASCs, i.e. pleiotropic genes that directly or indirectly affect the expression and activity of hundreds of downstream effectors, and whose functional alteration simultaneously affects diverse aspects of normal stem cell homeostasis. Genome-wide screens around MR genes have identified hundreds of their putative targets. However, the experimental validation of these bioinformatic predictions and the further characterization of putative downstream effectors as true stem cell regulators is notoriously lacking.

To begin addressing this gap between bioinformatic predictions and experimental validation, we use intestinal stem cells (ISCs) of the Drosophila melanogaster posterior midgut. We first integrated genome-wide DNA mapping data for two known MR genes in these cells: the transcription factor Escargot (Esg) and the signal transducer STAT. We then used qPCR analysis to test the response of over 80 predicted targets to the genetic manipulation of either or both MR genes in vivo, which allowed us to validate several of them as true targets. This analysis revealed a noticeable false discovery rate (FDR) with regards to mode of regulation by the MR genes among the bioinformatic predictions, as reflected by a higher than predicted rate of co-regulation by both Esg and STAT. Furthermore, the genetic manipulation of nearly all confirmed targets that we have tested thus far has resulted in noticeable phenotypes in ISC homeostasis, including changes to their number and/or morphology. Therefore, while our data would indicate that bioinformatic prediction of downstream target regulation by these MR genes is rather noisy, it is nonetheless highly predictive of a true ISC regulatory role for those targets that have been validated experimentally by independent means.

374A Sphingolipid metabolism regulates intestinal stem cell homeostasis *M. Mahidur Rahman*[1], Chenge Zhang[1], Marco Marchetti[1], Chloe Kraft[1], Collin Clark[1], William Holland[2], Scott Summers[2], Bruce Edgar[1] 1) Huntsman Cancer Institute, University of Utah; 2) Department of Nutrition and Integrative Physiology, Diabetes and Metabolic Research Center, University of Utah, Salt Lake City, Utah, USA

Sphingolipids are an essential component of cellular architecture. Sphingolipid metabolism regulates cellular processes such as proliferation, growth, apoptosis, inflammation, and senescence. Genetic defects in the metabolism of sphingolipids are associated with several metabolic disorders, including colorectal cancer. The molecular mechanisms

involved in sphingolipid metabolism-mediated intestinal stem cell (ISC) homeostasis and tumorigenesis are poorly understood. We do not know if and how the intermediate metabolites in the anabolic or catabolic processes of the ceramide metabolism regulate ISC fates in the adult midgut. To check if any of the sphingolipid metabolic enzymes are involved in the ISC homeostasis, we have genetically perturbed these enzymes in the specific cell types of the ISC lineages and the progenitor cells together. We found that the perturbation of different enzymes in the ceramide metabolism affects each cell type differently. Overall, the effects on the progenitors are manifested differently than those on the terminally differentiated enterocytes and enteroendocrine cells. For example, reducing ceramide synthases such as *lace* strongly affects enteroendocrine cell morphology compared to the ISCs or enteroblasts. Over-expression of the ceramidase *bwa* in the enteroblasts leads to robust proliferation of the ISCs, besides the increased size of the enteroblasts. On the other hand, the enterocytes are highly sensitive to any perturbation of the enzymes involved in the sphingolipid metabolism. Both increased or decreased levels of the enzymes kill the ECs, which in turn promote ISC proliferation. Since the EGFR signaling pathway is an important driver for ISC proliferation, we probed if the activated EGFR pathway would regulate the sphingolipid metabolism. Transcriptomic and lipidomic analysis of the Spitz activated EGFR pathway in the gut shows that the EGFR pathway can upregulate genes encoding ceramide biosynthesis as well as other sphingolipids. These results suggest that different enzymes and products of the sphingolipid metabolic pathway can act as signaling molecules and interact with major stem cell signaling pathways such as EGFR signaling to affect ISC homeostasis.

375B Identifying factors that maintain the adult testis niche *Gabriela Vida*, Elizabeth Botto, Stephen DiNardo
University of Pennsylvania

Stem cells are important for repairing and regenerating our tissues, and often reside in a niche that controls their behavior. The testis niche has been a paradigm for niche-stem cell interactions. Recently, our lab has focused on the construction of this niche. Examining initial assembly of the niche in embryonic gonads has revealed both how extra-gonadal signals drive niche assembly (Anllo and DiNardo, 2021), and begun illuminating the cell mechanics involved in first forming a compact, functional niche (B. Warder poster). 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 and the cyst stem cells. Our preliminary evidence suggests that acto-myosin contractility (AMC) is important for the maintenance of the spherical nature of the stem cell niche. The recently released single nuclear, RNA-SEQ Fly Cell Atlas (FCA) for the testis is a resource to suggest genes that might be candidates involved in niche structure. Center-divider (*cdi*) is suggested as enriched in the niche based on the FCA data and encodes a serine/threonine kinase known to induce actin remodeling through phosphorylation of cofilins. Our preliminary evidence shows that knockdown of *cdi* indeed affects maintenance of testis niche structure. We therefore hypothesize that the AMC and actin remodeling are both important for preserving niche structure and potentially critical for the maintenance of stem cells.

376C The role of ESCRTs in signaling within the testis stem cell niche *Mara Grace*, Erika Matunis Johns Hopkins University, Department of Cell Biology

Adult stem cells are crucial for regeneration, tissue repair after injury, and developmental processes such as spermatogenesis. Stem cells exist in a dynamic microenvironment termed the niche that provides signals to ensure the maintenance and self-renewal of the adult stem cell population. An appreciation of the dynamic communication between stem cells and their niche is vital to understand processes such as reproduction, oncogenesis, aging, development, and regeneration. Here I use the testis of *Drosophila melanogaster* as a model to investigate the role of endocytosis, and the ESCRT complexes specifically, in signaling within the stem cell niche. 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. Knockdown of ESCRTs in somatic stem cells causes niche cells to become significantly enlarged and exhibit abnormal morphology. Furthermore, Unpaired, a ligand for the JAK-STAT pathway normally secreted by niche cells, accumulates within the cytoplasm of niche cells upon ESCRT knockdown in somatic stem cells. As knockdown of ESCRTs in somatic stem cells affects the morphology of niche cells, this suggests that ESCRTs mediate signaling from somatic stem cells back to their niche to prevent niche hypertrophy. I am further exploring the role of additional pathways in modulating this signaling. A deeper understanding of the signaling dynamics within the *Drosophila* testis stem cell niche will have further implications for stem cell niches in other tissues and organisms as well as processes such as regeneration and renewal.

377A Drosophila Holes in muscles is required for ongoing adult muscle function and muscle stem cell maintenance. *Robert Hoff*, Richard Cripps San Diego State University

The Drosophila gene Holes in muscles (Him) is a mesodermal transcriptional corepressor that functions in myogenesis. Previously, we and others have characterized Him expression in the early embryonic mesoderm where it functions

downstream of known muscle specification genes as a repressor of the myogenic transcription factor MEF2 to delay muscle differentiation until the appropriate stage. Here, we extend our analysis of Him to other stages of Drosophila muscle development and function by analyzing the phenotype of the first-generated Him mutant allele. Him mutants show a reduction in the imaginal disc associated myoblast pool that form the adult thoracic muscles, including those required for jump and flight. Consistent with this observation, we find that Him mutants have reduced myonuclei in adult differentiated flight muscles. To further characterize the role of Him in adult muscle function, we assayed flight performance in young and aged flies. Him mutants perform comparably to wild type controls at young (3-5 days) of age, however have significantly reduced flight ability at two weeks of age, suggesting a role for Him in continued muscle maintenance. Additionally, we find that the flightless phenotype correlates with the number of somatic muscle stem cells associated with the flight muscles. Young Him mutants and control flies have a similar number of stem cells, but this number is significantly reduced in the mutants at two weeks of age. Altogether, these data suggest an ongoing role for Him in muscle beyond early development potentially mediated through regulating myonuclear number and stem cell maintenance.

378B Assessment of cellular and functional heterogeneity within the *Drosophila* testis stem cell niche *Jennifer Viveiros*, Erika Matunis Department of Cell Biology, Johns Hopkins University 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, and gaining insight into niche composition underpins our understanding of tissue homeostasis. Therefore, our ultimate goal is to further our understanding of stem cell niches by investigating their cellular composition and gene expression programs, using the *Drosophila* testis stem cell niche as a model. 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 use the HACK and Split-Gal4 ternary systems to genetically control individual populations of SGPs. Preliminary results suggest that combinatorial patterns of homeobox gene expression allow genetic dissection of SGPs arising from differing PS. These tools will allow lineage tracing and manipulation of the distinct populations of SGPs according to their origin to begin to determine if hub cell origin correlates with function, localization within the niche, and transcriptional program.

379C Investigating somatic stem cell cytokinesis and coordination of daughter cell release from the testis niche *Tiffany Roach*, Kari Lenhart Drexel University

Stem cells rely on instructional cues from their specialized microenvironment, or niche, to balance the production of self-renewing and differentiating daughter cells. Many adult tissues must coordinate the divisions and behaviors of multiple stem cell lineages to generate and maintain tissue. The *Drosophila* testis niche exhibits coordination between germline stem cells (GSCs) and cyst stem cells (CySCs) such that each differentiating GSC daughter must become completely encapsulated, or encysted, by two daughters of the CySC lineage. Our lab has found a modified cytokinesis program in GSCs that is critical for coordinated release of differentiating daughters. However, almost nothing is known about the cytokinesis program and cellular dynamics of the CySC lineage that may be acting in conjunction to facilitate this interaction. By establishing a combination of fluorescent markers to live image the CySC lineage, I will investigate CySC cytokinesis to determine the presence of a modified cytokinesis program that may be operating in tandem with GSC cytokinesis to enable coordinated release of stem cell daughters. Ultimately, this powerful new imaging system will permit us to identify the cell biology and temporal dynamics by which the 2:1 soma to germline ratio is established for tissue homeostasis. This work will extend our knowledge of the interactions and complex dynamics of two distinct stem cell populations within the testis niche.

380A Characterizing the Novel Protein Asperous Involved in Tissue Regeneration *Si Cave*, Robin Harris Arizona State University

The mechanisms of tissue regeneration are not fully understood. Using the model of the wing imaginal disc, we have identified factors upregulated during regeneration through RNA-sequencing. Asperous (CG9572) is a novel and mostly uncharacterized protein that is strongly transcribed specifically during regeneration. The goal of this work is to characterize Asperous in the context of regeneration, using a three-pronged approach to understand 1) its protein structure, 2) its localization, and 3) its cellular function in both damaged and undamaged imaginal disc tissues.

To do this, we have utilized computational tools to predict the structure and found the presence of a WD40 domain, suggesting a role in coordinating multi-protein complex assemblies. We have also used genetic manipulations via our unique genetic ablation tool, DUAL Control, to knockdown Asperous and have found it is essential for regeneration.

Finally, we are using fluorescent reporters of tagged Asperous proteins to understand its localization, which suggests it may be extracellular and contribute to differential growth in a compartment-specific manner. As Asperous has a uniquely regeneration-specific expression pattern and is associated with promoting regeneration in the wing disc, investigating the features of this novel protein will further our understanding of uncharacterized elements of a regeneration program.

381B Genetic determinants of cell fate plasticity during regeneration after radiation damage in *Drosophila* *Caitlin Clark*[1], Michelle Ledru[1,3], Jeremy Brown[1], Shay Segal[1], Shilpi Verghese[1,4], Sarah Ferrara[2], Andrew Goodspeed[2], Tin Tin Su[1,2] 1) Department of Molecular, Cellular and Developmental Biology, University of Colorado, Boulder, CO; 2) University of Colorado Cancer Center, Anschutz Medical Campus, Aurora, CO; 3) College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, CO ; 4) Department of Cell Biology, Emory University School of Medicine, Atlanta, GA

Ionizing radiation (IR) is used to treat approximately half of all cancer patients due to its ability to induce cell death. However, IR can also induce cancer stem cell-like properties in non-stem cancer cells, potentially promoting tumor regeneration and diminishing therapeutic success. Our published studies have shown that we can model IR-inducible stem cell-like properties in *Drosophila melanogaster* larval imaginal wing discs. After irradiation, IR-resistant cells from the hinge-region of the disc translocate to the pouch-region of the disc and change fate to help regenerate the pouch (Verghese and Su, 2016, PMID: 27584613; Verghese and Su, 2018, PMID: 30462636). Genome-wide RNAseq analysis of dissociated wing disc cells identified IR-induced gene expression changes specific to the hinge such as down-regulation of hinge determinants and up-regulation of Myc targets and ribosome biogenesis genes. Functional testing shows that Myc and genes that function in ribosome biogenesis are required specifically in the hinge for IR-induced fate change (Ledru et al., in revision for PLoS Genetics). Current efforts are directed at using hinge-specific ribosome profiling to identify mRNAs whose increased translation promotes cell fate change after irradiation.

382C Necrosis-induced apoptosis promotes regeneration in *Drosophila* wing imaginal discs *Jacob Klemm*, Robin Harris Arizona State University

Cell death is essential for the proper regeneration of tissues following injury. Dying cells serve as crucial signaling centers that promote cell proliferation and tissue remodeling following damage. Much of our understanding of these events comes from studies in *Drosophila* imaginal discs. For example, apoptotic cells release Wg and Mmp1, among other factors, to adjacent cells to promote tissue repair. This phenomenon been extensively characterized with respect to apoptosis-induced regeneration, however, much less is understood about how tissues respond to unregulated forms of death, like necrosis. Necrosis is a rapid, disordered cell death in which cell membrane integrity is lost and intracellular contents are released to the external environment. To study the regenerative response to necrotic tissue death, we developed a genetic ablation system that drives necrosis in the *Drosophila* wing imaginal disc.
With this new model, we have found that necrosis leads to a unique regenerative response. Immunofluorescent staining reveals significant apoptosis is induced at a distance from the wound, which we have termed necrosis-induced apoptosis (NiA). Unlike other damage-associated apoptosis in the wing disc, NiA cells are not regulated by the JNK pathway. Moreover, NiA cells are required for regeneration following necrosis; inhibition of NiA cell activity leads to lower levels of proliferation at the wound edge. Consequently, NiA cell inhibition results in a reduced capacity to regenerate, as assayed by adult wing size. This research shows that upon necrotic tissue death, wing discs rely on apoptotic signaling to facilitate regeneration.
We are currently focused on identifying the signals that lead to NiA cell activity and understanding the mechanism by which these cells promote proliferation. Preliminary data highlights cytokine and calcium signaling as potential regulators of NiA cells. Additionally, lineage tracing shows NiA cell progeny may contribute to the reconstruction of the *salm* domain. To comprehensively identify the mechanisms that define NiA cell activity, transcriptome data will be generated for NiA cells and wound edge regenerating cells. These data will characterize the tissue response to necrosis and provide insight to designing therapeutics for humans afflicted by necrotic injuries and diseases.

383A Elucidating The Roles of Zelda and Taranis During Late Regeneration in *Drosophila* Wing Imaginal Discs *Anish Bose*[1], Keaton Schuster[2], Rachel Smith-Bolton[1] 1) University of Illinois at Urbana Champaign; 2) New York University

Regeneration is a complex process that enables damaged tissues to be replenished and restored back to their correct morphology and function. As organs and tissues are prone to damage either by external forces or chronic illness, the identification of factors and mechanisms important for regeneration may have clinical significance. Our laboratory has described a regeneration-specific mechanism that stabilizes cell fate after damage, where the gene *taranis* maintains proper cell fate during late regeneration in *Drosophila* 3rd instar larval wing imaginal discs. Reduced Taranis levels allow damage-induced JNK signaling to overexpress *engrailed*. Overexpressed *engrailed* initiates a negative feedback loop that silences the *engrailed* locus, resulting in impaired patterning during regeneration, including posterior-to-anterior cell-fate transformations. Taranis prevents these posterior-to-anterior cell-fate changes by preventing *engrailed* overexpression. However, it is unclear which signals activate *taranis* expression during late regeneration, or how *engrailed* is regulated by JNK signaling or Taranis. Preliminary data showed that the pioneer transcription factor *zelda* was upregulated

during late regeneration, and in a reduced *zelda* background, *taranis* reporter expression was also reduced, suggesting that Zelda may be an activator of *taranis*. Furthermore, *zelda* may have additional roles beyond activating *taranis* in the regenerating wing imaginal disc. Finally, we are also examining how *engrailed* expression is misregulated by JNK signaling, and how that misregulation is prevented by Taranis.

384B The epithelial apical-basal polarity regulator Lgl constrains imaginal disc regeneration *Faith Karanja*, Rajan Bhandari, Thu Tran, Adrian Halme University of Virginia

Mutation of the neoplastic tumor suppressor *lethal (2) giant larvae (lgl)* in *Drosophila melanogaster* imaginal disc epithelia produces neoplastic tumors that elicit systemic responses, developmental delay, and growth inhibition, similar to those produced by regenerating imaginal discs. We observe that critical systemic and local regenerative signaling pathways are activated in *lgl*-mutant tumors, including expression of the relaxin-family hormone Dilp8, which is responsible for developmental delay and growth control during regeneration, and Wingless (Wg), which regulates growth and patterning in the wing during regeneration and normal development. Wg activation during regeneration is mediated by a downstream regulatory region, the damage responsive element (DRE), which we demonstrate is also responsible for Wg activation in *lgl*$^{-}$ tumors. We observed that removal of the DRE has little effect on *wingless* expression during normal disc development but severely reduces the regenerative capacity of imaginal tissues and prevents neoplastic transformation as well as neoplasia-mediated developmental delay. These observations led us to hypothesize that Lgl might function to limit regenerative activity that promotes the neoplastic transformation of discs. Supporting this hypothesis, we observed that: 1) inducing damage and regeneration in imaginal discs promotes the neoplastic potential of *lgl*$^{-}$ clones, which is blocked by removing the DRE, and 2) expression of a phosphorylation-resistant allele of Lgl strongly inhibits disc regeneration. Finally, during our analysis of *lgl*$^{-}$ clones in the wing imaginal disc, we found unexpected heterogeneity in the neoplastic potential and the DRE dependence between clones. This heterogeneity may reflect differences in regenerative pathways and potential found in different regions of the developing tissue. These results demonstrate an unexpected role for the epithelial apical-basal polarity regulator Lgl in regulating regenerative activity in imaginal discs.

385C Inducing limb regeneration in *Drosophila melanogaster* Michael Abrams[1, 3], Fayth Tan[1], *Yutian Li*[1], Ty Basinger[1, 5], Martin Heithe[1], Anish Sarma[1], Iris Lee[1], Zevin Condiotte[1], Misha Raffiee[1, 4], John Dabiri[2], David Gold[1, 6], Lea Goentoro[1] 1) Division of biology and Biological Engineering, California Institute of Technology, Pasadena, CA ; 2) Graduate Aerospace Laboratories and Mechanical Engineering, California Institute of Technology, Pasadena, CA; 3) Department of Molecular and Cell Biology, University of California at Berkeley; 4) Department of Bioengineering, Stanford University; 5) Department of Biology and Allied Health Sciences, Bloomsburg University; 6) Department of Earth and Planetary Sciences, University of California at Davis

Can limb regeneration be induced? *Drosophila* belongs to the holometabolan insects, which do not regenerate limbs or other appendages as adults. However, developing imaginal discs in larvae can regenerate, possibly suggesting an inherent ability to regenerate. In this study, we found that limb regeneration in adult flies can be induced. Strikingly, limb regeneration can be induced using, simply, nutrient supplementation with the amino acid L-leucine and the growth hormone insulin. Almost 50% the treated flies partially regrew their amputated limbs. The new leg part redeveloped cuticles and sensory bristles. Some flies completely reformed the amputated leg segment, followed by a joint and the beginning of next segment. This is the first demonstration that patterned regenerative response can be induced in adult *Drosophila* limb. Finally, the same strategy of leucine and insulin/sugar administration induce appendage regeneration in the moon jellyfish and digit regeneration in mice. These results suggest that latent regeneration is prevalent in animals and can be activated with relatively simple environmental stimuli. Our results present *Drosophila* as a novel system for studying how to induce regeneration.

Reference: MJ Abrams, FH Tan, Y Li, T Basinger, ML Heithe, AA Sarma, IT Lee, ZJ Condiotte, M Raffiee, JO Dabiri, DA Gold, L Goentoro. A conserved strategy for inducing appendage regeneration in moon jellyfish, *Drosophila*, and mice. eLife 2021;10:e65092 DOI: 10.7554/eLife.65092

386A Adapting the Nitroreductase Cell Ablation System to Drosophila *Gary Teeters*, Sarah Siegrist University of Virginia

Targeted cell ablation is a common method used to assay cell function during development and to determine cellular limits during regeneration. Many techniques are available to achieve cell ablation, yet improvements are still needed to increase spatial and temporal control. One method commonly used in the Zebrafish, *Danio rerio* is a Nitroreductase (NTR) mediated ablation system. In this technique cells expressing NTR can be induced to undergo cell death following drug treatment. For example, when metronidazole (MTZ) is exposed to cells expressing Nitroreductase, NTR converts MTZ into a DNA cross linker inducing cells to die. We set out to determine whether this commonly used cell ablation system could be used in Drosophila. We cloned the Nitroreductase gene from Escherichia Coli used in Curado et al. 2007 with a GFP reporter to examine cell death in vivo. This technique would allow for greater temporal control of cell ablation

without the need of temperature sensitive Gal80 or mutant alleles instead by regulating exposure to nitroimidazole antibiotics. This project means to adapt the protocols from *Danio rerio* to *Drosophila melanogaster* which will modify the exposure method, concentration, and time. We aim to show that exposure to nitroimidazole antibiotics through ingestion at sub toxic levels will be able to ablate cells in Gal4 specific manner with temporal control and no off-target toxicity.

387B The Role of *dMyc* in *Drosophila* wing imaginal disc regeneration *Felicity (Ting-Yu) Hsu*, Rachel Smith-Bolton University of Illinois at Urbana-Champaign

Regeneration is a complicated process through which some animals restore missing tissue upon damage. While many signaling pathways crucial for regeneration are critical in normal development, regeneration is not as simple as recapitulating normal development. We study regeneration using our genetic ablation system in *Drosophila* wing imaginal discs. One of the genes that are important in regeneration is *Myc*, whose expression levels and transcription levels are upregulated in the regenerating region of wing imaginal discs. Overexpression of Myc improves wing imaginal disc regeneration, while reduction of Myc expression worsens wing disc regeneration. During normal development, one primary role of Myc is ribosomal biogenesis. However, the exact function of Myc in regeneration is unknown. Our recent findings suggest that the nucleoli sizes, indicating ribosomal biogenesis activity, in the regenerating wing pouch are similar to those in the undamaged pouch. Interestingly, smaller nucleoli were found in the undamaged hinge tissue of the damaged wing discs. This finding suggests that one possible role of Myc might be maintaining ribosomal biogenesis activity in the regeneratiion blastema while the activity is reduced in other parts of the damaged disc. Thus, we aim to identify the exact role of upregulated Myc in wing disc regeneration. In addition to ribosomal biogenesis, Myc is also involved in cell competition. After tissue damage, the difference in Myc expression levels between the regeneration blastema and the rest of the disc is increased, as Myc is only upregulated in the regenerating tissue. During normal development, Myc expression level differences in the wing imaginal disc lead to cell competition, where cells with lower Myc are eliminated. However, we observed no extreme cell death at the boundary between high and low Myc expression levels in regenerating wing discs. Therefore, we aim to study whether cell competition takes place in regenerating wing discs.

388C Wear and Tear of the Intestinal Visceral Musculature by Intrinsic and Extrinsic Factors *Ho Kim*[1], Eric So[1], Jiae Lee[1], Yi Wang[2], Vikram Gill[1], Anna Gorbacheva[1], Hee Jin Han[1], Katelyn Ng[1], Ken Ning[1], Inez Pranoto[1], Alejandra Cabrera[1], Dae Seok Eom[2], Young Kwon[1] 1) University of Washington; 2) University of California Irvine

The gut visceral musculature plays essential roles in not only moving substances through the lumen but also maintaining the function and physiology of the gut. Although the development of the visceral musculature has been studied in multiple model organisms, how it degenerates is poorly understood. Here, we employ the *Drosophila* midgut as a model to demonstrate that the visceral musculature is disrupted by intrinsic and extrinsic factors, such as aging, feeding, chemical-induced tissue damage, and oncogenic transformation in the epithelium. Notably, we define four prominent visceral musculature disruption phenotypes, which we refer as ‘sprout’, ‘discontinuity’, ‘furcation’, and ‘crossover’ of the longitudinal muscle. Given that the occurrence of these phenotypes is increased during aging and under various stresses, we propose that these phenotypes can be used as quantitative readouts of deterioration of the visceral musculature. Intriguingly, administration of a tissue-damaging chemical dextran sulfate sodium (DSS) induced similar visceral musculature disruption phenotypes in zebrafish larvae, indicating that ingestion of a tissue-damaging chemical can disrupt the visceral musculature in a vertebrate as well. Our study provides insights into the deterioration of the gut visceral musculature and lays a groundwork for investigating the underlying mechanisms in *Drosophila* as well as other animals.

389A Transition from acute nerve injury to central sensitization requires metabotropic driven astrocyte store-operated Ca^{2+} entry *Mariya Prokhorenko*, Jeremy Smyth Uniformed Services University of the Health Sciences

Chronic pain following injury is a debilitating condition associated with serious comorbidities and opioid dependence. Understanding the neurological mechanisms that underlie the transition from initial injury to chronic pain is essential for prevention and treatment. This transition is mediated by central sensitization, which involves reversible changes to nociceptive neurocircuitry pathways in the central nervous system. Central sensitization research and therapeutics have focused on neuronal circuitry, whereas the roles of glial cells are poorly defined. Notably, astrocytes that normally maintain and modulate neuronal synapses become reactive and exhibit aberrant Ca^{2+} signals during central sensitization. However, how these Ca^{2+} signals are generated and how they modulate astrocyte function in central sensitization are unknown. Importantly, central sensitization involves significant upregulation of glutamate release at nociceptive synapses, and glutamate acts on astrocytes via metabotropic glutamate receptors to stimulate Ca^{2+} release from endoplasmic reticulum (ER) Ca^{2+} stores via inositol 1,4,5-tripshosphate receptors (IP_3R). We are testing the hypothesis that central sensitization requires astrocyte metabotropic signaling involving IP_3R-mediated Ca^{2+} release from ER Ca^{2+} stores and activation of store-operated Ca^{2+} entry (SOCE). We are testing this by combining powerful genetic tools with a robust model of central pain sensitization in *Drosophila melanogaster*. We assay central sensitization in flies by

amputating a single leg and monitoring the response to subnoxious temperatures. Injured, sensitized animals exhibit increased jumping behavior at the subnoxious temperature, whereas animals without injury do not respond to this temperature. Using a combination of fluorescence imaging techniques, we show enhanced astrocyte Ca^{2+} signaling in the ventral nerve cord precedes behaviorally measured central sensitization after injury, including changes in live signaling using GCaMP and cumulative signaling using Transcriptional Reporter of Intracellular Calcium (TRIC). We also found that astrocyte-specific suppression of IP_3R as well as the SOCE components Stim and Orai attenuates nociceptive jumping behavior at one week following nerve injury, suggesting a key role for IP_3R and SOCE-mediated Ca^{2+} signaling in astrocytes during pain sensitization. Our results will bring new understanding to the role of astrocyte signaling in pain sensitization and may suggest novel therapeutic targets for the prevention or treatment of chronic pain.

390B Wound-induced changes in epithelial tension *Ivy Han*, James White, James O'Connor, M. Shane Hutson, Andrea Page-McCaw Vanderbilt University, Nashville, TN, USA

Understanding the mechanisms of epithelial wound healing is a major challenge in biology and medicine. *Drosophila* is a great model to study wound healing and has contributed significantly to identifying how epithelial tissue repairs a wound through coordinated changes in cellular behavior (e.g., cell migration). Wound-induced epithelial cell migration is preceded by dynamic changes in cytoskeletal structures and in tissue tension. However, it is unclear how epithelial wounding immediately affects tension in nearby epithelial cells or whether changes in tension play a role in the wound response. This study aims to characterize how wounding alters the cortical tension of apical cell borders in epithelial cells and determine factors that modulate these wound-induced tension changes. We use a laser-recoil assay to measure cortical tension immediately after a wounding the *Drosophila* pupal notum epithelium, and controls expressing Rok-RNAi confirmed that the technique could identify cells with reduced cortical tension. We discovered that within minutes after wounding, tension was reduced in epithelial cells closer to the wound in a distance-dependent manner. Wounds were administered by a single-shot pulsed-laser ablation, which causes a cavitation bubble that damages cells around the wound. Using a different method of laser-wounding, we found that plasma membrane microtears from the laser-induced cavitation bubble were necessary for reducing cortical tension near the wound. Previously, our group identified that the G-Protein Coupled Receptor (GPCR) Methuselah-like 10 (Mthl10) is required for calcium signaling within about a minute after wounding. Our preliminary results show that knockdown of *mthl10* alters tension around the wound, abolishing the distance-dependent tension reduction after single-shot pulsed-laser wounding. This suggests that Mthl10 may be a key factor that modulates wound-induced tension changes, either by preventing tension loss or restoring tension at locations distal to the wound. Further investigation on connections between epithelial tension and other established wound responses such as calcium signaling, cell fusion, and cell migration could pave the way for deeper understanding of the mechanobiology of epithelial wound healing.

391C The Role of Polyploidy During *Drosophila* Epithelial Wound Repair *James White*, Kimi LaFever Hodge, Ivy Han, Jasmine Su, M. Shane Hutson, Andrea Page-McCaw Vanderbilt University

In the past decade, there has been increased awareness of cellular polyploidy in development, homeostasis, and cancer. In addition to being a conserved developmentally programmed behavior, polyploidy is also induced in response to injury. Work by Vicki Losick has shown adult *Drosophila* epithelia repair by becoming polyploid through endoreplication and cell-cell fusion. In Zebrafish epicardial explants, regeneration is led by a wavefront of polyploid cells. We have found that both endocycling and cell fusions occur after laser wounding in the pupal notum, a system that supports live imaging. By imaging living tissue, we find that syncytia are able to close wounds faster than non-syncytial neighboring cells, and when syncytia formation is blocked, wounds close more slowly. Live imaging also allowed us to determine that cell-cell fusions occur within the first 20-30 minutes after wounding, and cell borders break down within the first 3 rows of cells from the wound. Thus, multinuclear syncytia form very rapidly and are positioned at the front lines of wound repair. The majority of fusion events occur between cells at different distances from the wound, as opposed to neighbors in the same row. Given this new spatial-temporal information I hypothesize fusions of sequential rows of cells allows distal cellular resources to be pooled at the leading edge of wound repair allowing for the construction of large actin-rich contractile structures. Preliminary live-imaging studies of actin support this hypothesis, as actin label in fused cells aggregates at the wound margin in large filipodia like structures. These large structures likely allow for rapid migration during the early stages of wound repair.

392A Insulin receptor/Akt/TOR signaling regulates muscle stem cell pool in Drosophila *Kumar Vishal*, Richard Cripps San Diego State University, San Diego, CA

Indirect flight muscles (IFMs) are the largest muscles in Drosophila and are made up of hundreds of myonuclei. The generation of these giant muscles requires a large pool of wing disc associated muscle stem cells. To achieve this, there is an activation of quiescent muscle stem cells followed by a rapid scaling up of the pool in the postembryonic stages, where the number of adult muscle stem cell increases from 10-20 to 2500 within 4 days. However the factors that control activation and proliferation to form this muscle stem pool are incompletely known. The goal of this study is to examine the role of Insulin receptor/Akt/TOR signaling in the regulation of muscle stem cell. We find that blocking the

components of Insulin receptor/Akt/TOR signaling results in a diminished myoblast pool. This reduction in the pool size is due to decreased muscle stem cell activation and proliferation. Disrupting the Insulin receptor/Akt/TOR signaling at initial stages of larval development results in an absence of myoblast pool activation. On the other hand, abrogating signaling in the late larval stage reduces muscle stem pool. Furthermore, our results demonstrate that the attenuation of the Insulin recptor signaling leads to complete absence of IFM formation. By contrast, activating the pathway increases the pool size and the proliferative capacity of the muscle stem cells. Finally our genetic interaction data suggests that Akt interacts with a pseudokinase Tribbles to regulate muscle stem cell population. Collectively, our studies identify a novel role for Insulin receptor/Akt/Tor signaling in the activation and proliferation of the muscle stem cell pool.

393B Cell cycle exit and stem cell differentiation are coupled through regulation of mitochondrial activity in the *Drosophila* testis *Diego Sainz de la Maza*[1], Silvana Hof-Michel[2], Lee Phillimore[1], Christian Bökel[2,3], Marc Amoyel[1] 1) University College London, UK; 2) Philipps University Marburg, Germany; 3) Universität Ulm, Germany

Stem cells maintain tissue homeostasis by proliferating to replace cells lost to damage or natural turnover. To do so, they need to balance self-renewal and differentiation. Whereas stem cells proliferate, terminal differentiation is accompanied by exit from the cell cycle, so we asked how cell identity was coordinated with cell cycle exit. We use the *Drosophila* testis as a model. The testis niche supports two stem cell populations, germ line stem cells and somatic cyst stem cells (CySCs) by providing signals necessary for their self-renewal. CySCs proliferate while their differentiating daughter cells do not, providing an ideal model to ask how stem cell identity is linked to proliferation. We show that Cyclin E is required for CySC self-renewal and blocking the G1/S transition causes premature differentiation. Conversely, knocking down Rbf, the homologue of Retinoblastoma that inhibits the G1/S transition, expanded the CySC population and blocked its differentiation. Rbf functions by inhibiting the complex E2f1/Dp. Surprisingly, E2f1/Dp activity was not required for self-renewal, suggesting that the endogenous role E2f1/Dp activity is not in promoting cell cycle progression but to prevent differentiation in cycling cells. To determine how Rbf inhibited differentiation, we analysed gene expression upon Rbf knockdown. Genes regulating mitochondrial biology and energy production were downregulated in Rbf-deficient testes. Promoting mitochondrial biogenesis and activity in Rbf knockdowns by expressing the PGC1α homologue, Spargel (Srl) and Ets97D/NRF-2 rescued differentiation of CySCs. However, we observed ectopic cycling in rescued, differentiated cells suggesting that oxidative metabolism is essential for CySCs differentiation but is not sufficient to exit the cell cycle. Thus, our results indicate that Rbf coordinates cell cycle exit and differentiation by inhibiting E2f1/Dp and promoting a metabolic state that is permissive for differentiation.

394V Molecular mechanisms behind adult muscle stem cells specification and activation. *Hadi Boukhatmi*[1, 2, 5, 6], Nourhene Ammar[1, 2], Sarah Bray[5, 6], Jennifer Zannet [3, 4], François Payre[3, 4] 1) Institut de Génétique et Développement de Rennes, CNRS, Rennes ; 2) Université de Rennes 1 ; 3) Molecular, Cellular and Developmental Biology Department (MCD), Centre de Biologie Intégrative (CBI), CNRS, Toulouse ; 4) Université Paul Sabatier ; 5) University of Cambridge ; 6) Department of Physiology, Development and Neuroscience.

To compensate for damages and cell death, adult tissue homeostasis requires regeneration operated by long-lived stem cells. Skeletal muscles are regenerated by muscular stem cells called satellite cells (MuSC), which are quiescent and, upon injury, become activated and differentiate into myoblasts to repair muscles. A key question is how such cells are specified and then protected from differentiation for a prolonged developmental period. We discovered that MuSC maintenance involves persistent expression of the Zfh1/ZEB Transcription Factor (TF). We showed that sustained Zfh1 expression, in MuSC requires production of an alternate *zfh1* RNA isoform, which is insensitive to microRNAs (*miR8/miR200*) degradation. Zfh1 protein is thus maintained in these cells, enabling them to escape differentiation and persist in the adult and contribute to muscle homeostasis. The key role of ZEB1 in the control of mammalian MuSCs maintenance has been recently reported, showing the broad relevance of *Drosophila* toward a better understanding of MuSC regulation. While MuSC specification and differentiation starts to be well understood, little is known about the genetic control that underlies the early steps of their activation. Our results revealed that another conserved TF, OvoL/Shavenbaby (Svb), is expressed in adult MuSCs. Svb is the unique *Drosophila* member of the OvoL family of TFs, which are emerging as key regulators of epithelial tissues and stem cells. We found that the Svb TF binds *in vivo* to *zfh1* cis-regulatory regions suggesting a direct regulation of *zfh1* transcription by Svb. Interestingly, *svb* is rapidly turned off in new-born differentiating progenies that replenish the muscle fibre. Upon *svb* loss of function in adult MuSCs, we observed dramatic increase in the rate of myogenic progenitor production indicating that Svb controls the frequency of MuSC division rate. Building on these data, we concluded that Svb have a dual role in MuSCs: **a)** Maintaining stemness through *zfh1,* **b)** Acting as an early activating gene by regulating the rate of MuSC division.

395V Regulation of Damage-Responsive Maturity-Silenced enhancers in *Drosophila* *John Quinn*, Robin Harris Arizona State University

Regeneration is a complex process that occurs in a variety of different organisms. In early larval stages, the imaginal discs of *Drosophila* – the precursors to the adult appendages – have a significant capacity to regenerate that is lost as the organism matures. Using a genetic ablation method established by our group we have found that several genes involved

in disc regeneration are regulated by damage-responsive and maturity-silenced (DRMS) enhancers. These regulatory elements are activated upon damage to induce regenerative gene expression, but epigenetically silenced as the organism approaches pupariation. The goal of my research is to investigate what specific signals activate DRMS enhancers and how they become progressively silenced as discs mature.
The genes *wg* and *Wnt6* are activated during regeneration and controlled by a single DRMS enhancer ($DRMS^{Wnt}$). We found that JNK signaling is necessary for the activation of $DRMS^{Wnt}$ but not sufficient, since developmental JNK signaling does not activate the enhancer. The cells contributing to regeneration co-express both JNK and JAK/STAT consistent with previous data that shows these pathways are important for regeneration. Using a GFP reporter for $DRMS^{Wnt}$ we found that reducing JAK/STAT pathway activity via STAT92E RNAi decreases damage-induced activation of the reporter, suggesting a role for JAK/STAT in the activation of the enhancer. Although, this decrease indicates that JAK/STAT is involved in regeneration, it does not show whether STAT is directly interacting with the enhancer DNA. To test this interaction, we generated a $DRMS^{Wnt}$-GFP lacking the consensus transcription factor binding sites to STAT92E, which shows a similar reduction in GFP to that seen with STAT RNAi. To understand the dynamics of how JAK/STAT activates DRMS we examined how a reduction in STAT might affect regeneration over a 48hr period. Our results show that STAT is required for full enhancer activity and does not preferentially work at a certain point of regeneration.
Going forward, we are overexpressing the JAK/STAT pathway elements to determine the necessity and sufficiency in DRMS enhancer activation and whether it can improve regeneration. Furthermore, we are also testing whether this genetic relationship is relevant to other DRMS enhancers that we have identified.

396V Switching On/Off the Hh signalling Pathway Determines Niche Cell Fates of Ovarian Germline Stem Cells *Yu-Ting Wang*[1,2], Chun-Ming Lai[3], Hwei-Jan Hsu[1,2] 1) Academia Sinica, Taipei City, Taiwan; 2) National Defense Medical Center, Taipei City, Taiwan; 3) Louisiana Cancer Research Center, Tulane University, LA, USA

The balance between stem cell renewal and differentiation is critical for tissue homeostasis, and that is largely regulated by stem cell microenvironments, or niches, formed by specific groups of cells. The stem cell maintenance niche recruits and maintains stem cell pool, while the stem cell differentiation niche promotes stem cell differentiation. However, little is known about how these niches are formed. Here, we report that off and on states of Hh signaling determine the fate of maintenance (cap cells) and differentiation niche (escort cells), respectively, of the Drosophila ovarian germline stem cell (GSC). During development, Hh signaling is activated in intermingled cells (ICs, niche precursors), while it is absent in cap cells but remained in escort cells. Suppressing Hh signaling turnover in ICs, results in cap cell reduction, and induces cap-escort transitional cells that eventually shift toward the escort cell lineage. We also report that Hh signaling in cap cells is silenced via Notch signaling and Cullin3 (Cul3)-HIB-mediated proteasome in a parallel or an upstream-downstream manner. From a small-scale RNAi screening for Cul3 regulators combined with the in silico promoter analysis, we find that the promoter of Ubc10, an E2 ubiquitin-conjugating enzyme, carries two putative Notch responsive elements, and Ubc10 knockdown in ICs phenocopies the above-described phenotypes. Interestingly, Ubc10 with its E3 partner, Ari-1 (Ari-1-Ubc10) is proposed to coordinate with Cul3-HIB complex for protein degradation, an E3-E3 tagging model. Although more studies are needed to pinpoint the involved mechanisms, our studies have added knowledge on the establishment of stem cell niches, and that may be applied to other stem cell systems.

398V The role of *Diaphanous* in the reactivation of quiescent neural stem cells *Kun-Yang Lin*[1], Wei Yung Ding[1], Hongyan Wang[1,2] 1) Neuroscience & Behavioral Disorders Programme, Duke-NUS Medical School, Singapore, ; 2) Department of Physiology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore

The switch between quiescence and proliferation of stem cells is critical for tissue development and homeostasis. The failure of neural stem cell reactivation is associated with neurodevelopmental disorders, such as microcephaly. Drosophila neural stem cells (NSCs) have emerged as an ideal *in vivo* model for studying quiescence and reactivation. Previously, we discovered that the primary protrusion, a hallmark of quiescent NSCs, is enriched for the actin cytoskeleton. However, the role of the actin cytoskeleton and its regulators have not been established in quiescent NSCs. Here, we report that Diaphanous (Dia), an actin regulator promoting actin polymerization, regulates F-actin polymerization and reactivation of quiescent NSCs. *dia* mutants show delayed NSC reactivation and small brain phenotype. Depletion of *dia* via RNA interference in the quiescent NSCs causes a reactivation delay and the reduction of Filamentous actin. Moreover, overexpression of constitutively active form of Dia leads to the delay of NSC reactivation and larval growth, while overexpressing wild-type form of Dia does not affect NSC reactivation. Together, our results suggest that Dia regulates actin polymerization and is a novel regulator for the reactivation in quiescence NSC.

399V Regulation of nutrient-independent proliferation of the mushroom body neuroblasts (MB NBs) in Drosophila melanogaster *Md Ausrafuggaman Nahid*[1], Conor Sipe[2], Sarah Siegrist[1] 1) University of Virginia; 2) Shepherd University

Drosophila neural stem cells (NSC), known as neuroblasts (NB), undergo asymmetric cell division throughout development in order to make the adult fly brain. Dietary nutrients provide essential building blocks necessary for NB growth and proliferation. However, there is a subset of NBs, known as mushroom body neuroblasts (MB NBs), which are able to continue proliferation regardless of extrinsic dietary nutrient availability. My research aims to understand the

molecular mechanism regulating nutrient-independent MB NBs proliferation. Transcriptional co-activator Yorkie (Yki) is well known for its role in maintaining tissue growth and size. However, it remains unclear whether Yki controls the nutrient-independent proliferation of the MB NBs. Upon NB specific Yki knockdown, MB NBs stopped proliferation in response to dietary nutrient withdrawal and resumed proliferation upon re-feeding. Upon expression of a constitutively active form of Yki, all NBs in the fly brain continued cell proliferation independent of dietary nutrient availability. This suggest that Yki regulates NB proliferation decision in response to nutrient availability. Yki requires a DNA binding partner for its function and Sd is the most well-known in *Drosophila*. I knocked down Sd in a NB specific manner and it did not stop MB NBs from proliferating in a nutrient-independent manner which suggests that Sd might not be the Yki binding partner in the brain. NB specific knockdown and over-expression of Myc resulted in a similar phenotype as Yki. I will conduct two experiments to determine whether there is a genetic interaction between Myc and Yki in the MB NBs. I will over-express Myc in Yki knockdown NBs, and also over-express Yki in Myc knockdown NBs.

400V Consequences of monosomy: How stem cells can lose their female identity and start tumors. *Annabelle Suisse*, Lara Alzouabi, Allison Bardin Institut Curie, PSL Research University, CNRS UMR 3215, INSERM U934, Stem Cells and Tissue Homeostasis Group, Paris, France.

Aneuploidy, the loss or gain of chromosomes, is the most prevalent genetic hallmark of cancer. However, in a normal context, it is difficult to assess its frequency and its impact as aneuploid cells are often eliminated. It is not known whether chromosome gain or loss happens in healthy stem cells, whether this changes during aging, or how stem cells cope with it. Here, we use the Drosophila intestinal stem cell model to explore these questions.
Our previous data from whole genome sequencing in aged female Drosophila midguts have revealed a loss of an entire X chromosome (monosomy), suggesting that stem cells in healthy tissue can undergo aneuploidy. We have developed a genetic system to study this further, based on the loss-of-heterozygosity (LOH) of an X-linked tumor suppressor gene, whereby loss of the wild-type X chromosome results in neoplastic growth. Using a specific marker, we confirmed that loss of an X chromosome occurs frequently (1 in 800 stem cells).
How do these cells survive and compensate for the sudden loss of an entire chromosome?
We believe that part of the answer lies with buffering by the dosage compensation (DC) pathway, which in male cells deposits H4K16ac on the single X and increases its transcription. We show that DC becomes activated in female monosomic cells, indicating for the first time that a X chromosome counting mechanism still exists in adult cells. We are now testing whether DC is required in monosomic cells for neoplasia formation and effectively buffers the loss of one X.
Our data suggest that another factor promoting the survival and fitness of aneuploid cells could be loss tumor suppressor activity. Indeed, X chromosome monosomy is more frequent when a tumor suppressor is inactivated than in a wild-type context. This suggests that the inactivation of tumor suppressor genes could foster an environment where aneuploid cells can survive and thrive.
Together, our work shows that monosomy happens frequently in healthy stem cells, is an important mechanism of LOH and can be viable, making it a driving force of tumorigenesis.

401V Role of the PIWI protein Aubergine in the regulation of intestinal regeneration *Karen Bellec*[1], Lynsey R Carroll[1], Yu Yachuan[2], Rippei Hayashi[3], Julia Cordero[1,2] 1) University of Glasgow; 2) CRUK Beatson Institute; 3) Australian National University

Adult stem cells are essential actors in the maintenance of basal homeostasis and damage-induced regeneration in self-renewing tissues. This relies on an accurate regulation of the proliferation and differentiation of stem cells by highly conserved and coordinated signaling pathways. Consistently, disorders in signaling activity may lead to homeostasis disruption inducing age-associated tissues dysfunctions as well as a wide range of cancers.
One of the interests of our laboratory is to identify and understand the molecular mechanisms controlling and adjusting intestinal stem cell (ISCs) behavior to fit the proliferative demands of the gastrointestinal epithelium. For this, we work with the adult *Drosophila* intestine, an excellent model system that shares functional and structural homologies with its mammalian counterpart. Previous pioneer works shed light on multiple evolutionarily conserved signaling pathways involved in the regulation of ISCs proliferation such as Wnt signaling, JAK/STAT or EGFR signaling. Unexpectedly, the Piwi-interacting RNA pathway (PIWI pathway), known for its role in the repression of transposable elements in the germline, has also been shown to be essential for ISCs maintenance and function through the repression of transposable elements (Sousa-Victor et al., 2017).
Interestingly, we discovered that Aubergine (Aub), another component of the PIWI pathway, is specifically expressed in ISCs and required for their proliferation upon intestinal damage. The abolition of stem cell proliferation upon knockdown of other PIWI pathway components point to a canonical role of Aub in intestinal regeneration. This is consistent with our small RNA sequencing data revealing an increase of piRNA in the intestine upon damage which is abolished upon the knockdown of Aub. In the *Drosophila* germline, Aub interacts with the translation initiation factor eIF3 to promote target gene translation. In the intestine, this interaction is conserved: eIF3 is expressed in ISCs upon intestinal damage and the loss of Aub is associated with a decrease of eIF3C protein. In addition, the knockdown of eIF3 recapitulates the

phenotypes observed upon the loss of Aub.
Altogether, our experiments reveal a new mechanism involved in the regulation of stem cell behavior. Our work bears implications for regenerative medicine, ageing and cancer research as it may lead to the identification of new therapeutic targets to prevent stem cell dysfunction.

402V WD40 Wuho regulates intestinal stem cell homeostasis for Gut integrity and Longevity *Kreeti Kajal*[1,2,3], Hwei-Jan Hsu[1,2,3] 1) Molecular and Biological Agricultural Sciences Program, Taiwan International Graduate Program, National Chung Hsing University and Academia Sinica, Taipei; 2) Graduate Institute of Biotechnology, National Chung Hsing University, Taichung; 3) Institute of Cellular and Organismic Biology, Academia Sinica, Taipei, Taiwan

Animal health and longevity require proper maintenance of gut homeostasis, which is largely supported by intestinal stem cells. However, the genetic regulators of stem cell activation and differentiation remain poorly understood. Here, we report that Wuho (Wh, a conserved WD40 protein) maintains the balance between intestinal stem cell (ISC) proliferation and differentiation, which affects the intestinal epithelial barrier and longevity in *Drosophila* females. Wh is enriched in ISCs; mutation of *wh* or knockdown in ISCs leads to ISC over-proliferation and aberrant differentiation of ISCs toward absorptive enterocytes. Furthermore, *wh* mutants exhibit leaky gut and upregulation of antimicrobial peptide transcripts, partially accounting for the shortened lifespan of *wh* mutants. In addition, ROS levels and JNK signaling are increased in the ISC lineage of the *wh* mutant gut, possibly due to bacterial infection. Elevated ROS and JNK signaling are known to enhance ISC proliferation and cause gut dysplasia. Thus, Wh appears to intrinsically and extrinsically regulate ISCs in order to promote gut homeostasis. Surprisingly, *wh* mutant ISCs, generated by FLP/FRT-induced mitotic recombination, show limited proliferation and differentiation capacities, suggesting a role of Wh in keeping ISCs in a quiescent state within the young gut. We speculate that when Wh is dysfunctional in all ISCs, a compensatory mechanism may be triggered to promote ISC proliferation, resulting in gut dysplasia. Furthermore, Wh expression is decreased in aged ISCs, and *wh* mutant guts mimic the aged gut phenotype, suggesting that Wh maintains gut physiological aging. We are currently working to identify Wh-interacting partners in the ISC lineage. Nevertheless, our results thus far suggest that Wh may prevent age-associated intestinal dysplasia and inflammation.

ICOB intramural funding supports this work.

403V Kinetics of blood cell differentiation during hematopoiesis revealed by quantitative long-term live imaging *Kevin Ho*[1], Rosalyn Carr[2], Alexandra Dvoskin[1], Guy Tanentzapf[1] 1) Department of Cellular and Physiological Sciences, University of British Columbia, Vancouver, Canada, V6T 1Z3; 2) School of Biomedical Engineering, University of British Columbia, Vancouver, Canada, V6T 1Z3

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 organ culture and imaging strategy for hematopoiesis in flies that takes advantage of powerful genetic and transgenic tools available in this system. We find that fly blood progenitors undergo self-renewal, suggesting that they are true stem cells. Using quantitative imaging to simultaneously track markers for stemness and differentiation in progenitors, we identify two types of differentiation that exhibit distinct kinetics. Moreover, we find 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, aggregate effects to transform stem cells from a quiescent to an activated state.

404V Enteroendocrine control of intestinal health and disease in Drosophila *Andre Medina*[1,2], Julia Cordero[1,2] 1) Cancer Research UK Beatson Institute; 2) University of Glasgow

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, which are translated into major physiological outputs. As in vertebrates, *Drosophila* has a diverse EE system scattered along the midgut epithelium and organized into subpopulations of cells expressing different neuroendocrine (NE) peptides. EE cells and NE peptides alterations are recognized features of intestinal damage and gastrointestinal pathologies, such as inflammatory bowel diseases (IBD). However, the functional significance of EE cell disruptions in intestinal physiopathology remains poorly understood. Here, we used adult *Drosophila melanogaster* as an in vivo model organism to study EE cells function and the functional implications of EE cell disruptions in intestinal damage and inflammation. Previous studies have shown the importance of EE cells to maintain intestinal homeostasis. Depletion of EE cells has a strong impact on intestinal regeneration. On the other hand, we have observed that long-term damage or inflammation favors the increase of EE cell proportion and ectopic expression of NE peptides. To unravel the functional significance of this phenomenon, we performed a genetic screen to identify gut-derived NE peptides that impact ISC proliferation. Our results suggest a novel role for the EE peptide DH31 in this context. DH31 expression in the adult midgut is upregulated upon intestinal damage by feeding with the bacteria *Pseudomonas entomophila* or chemical treatment with ROS, resulting in previously unrecognized signaling

to midgut-associated tissues and regulation of ISC niche factors to support intestinal regeneration.

405V JNK and JAK/STAT stratify cell behaviors during tissue regeneration *Janhvi Jaiswal*[1,2], Raphael Engesser[3], Andrea Armengol Peyroton[1], Carlo Crucianelli[1], Isabelle Grass [1,5], Jens Timmer [3], Anne-Kathrin Classen[1,4,5] 1) Hilde Mangold Haus, University of Freiburg; 2) Spemann Graduate School of Biology and Medicine (SGBM), University of Freiburg; 3) Institute of Physics, University of Freiburg; 4) CIBSS Centre for Integrative Biological Signalling Studies, University of Freiburg; 5) BIOSS Centre for Biological Signalling Studies, University of Freiburg

Epithelia are frequently exposed to extracellular insults and damage due to their exposed position. The restoration of homeostasis upon damage relies on regulated activation of complex stress-responsive signaling pathways to coordinate cellular responses such as apoptosis, G2 stalling, proliferation and survival. Consequently, deviations from these coordinated responses, due to prolonged or potent damage, lie at the center of chronic wound pathologies. Despite their importance, the understanding of how spatial patterns of signaling pathways guide repair behaviors, how the extent of damage is matched by a response of appropriate duration and strength, and how precisely these processes become deviated within damaged tissues remains elusive. Using TNF-α/Eiger-mediated damage to the wing imaginal disc of *Drosophila*, we observe a stratification of cellular responses coinciding with the spatial patterns of the JNK and JAK/STAT pathways. We find a distinct cell-autonomous repression of JAK/STAT by JNK, and demonstrate through mathematical modeling the importance of such repression to generate our observed spatial patterns. Importantly, JNK-dependent G2 stalling is overridden by ectopic expression of JAK/STAT, leading to increased apoptosis in the tissue. This suggests that the repression of JAK/STAT by JNK is essential to stratify distinct cellular repair behaviors at the wound site. Understanding how perturbations to these patterns impair regenerative responses would be a step towards creating new perspectives into previously uncharacterized links between important signaling pathways and their underlying repair behaviors - which fundamentally influence wound healing pathologies.

406V Imaginal disc regeneration: from stress to nutrients *José Esteban Collado*[1], Montserrat Corominas[1,2], Florenci Serras[1,2] 1) University of Barcelona; 2) Institute of Biomedicine of the University of Barcelona (IBUB)

Regeneration is the ability to rebuild a body part that has been damaged or amputated. *Drosophila* imaginal discs are able to undergo wound healing and regenerative growth after injury or genetic ablation. Reactive oxygen species (ROS) act as early signals that are sensed by the MAP3 kinase Ask1, which in turn activates downstream by phosphorylation the MAP kinases p38 and JNK. Apoptosis can occur as the result of sustained or high activation of these kinases, whereas short or low activation can promote regeneration. In search for the conditions that cells require to activate Ask1-dependent regeneration program we find that PI3K/Akt signaling is necessary for Ask1 to activate p38, but not JNK. In addition, nutrient restriction or mutations that target Ser83 of the *Drosophila* Ask1 protein, a PI3K/Akt-sensitive residue, impairs regeneration. However, these effects can be reversed by the ectopic activation of p38, but not of JNK. Our results demonstrate that the phosphorylation of p38 during regeneration is nutrient sensitive, and that Ask1 controls the activation of p38 through Ser83. This mechanism is important for discriminating between p38 and JNK signaling pathways in the cells involved in tissue repair and regenerative growth.

407V Ets21C organizes a pro-regenerative microenvironment that is essential for imaginal disc regeneration *Melanie Worley*, Nicholas Everetts, Riku Yasutomi, Nir Yosef, Iswar Hariharan University of California, 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.

408B Functional dissection of recently diverged HMG-box proteins in Drosophila spermatogenesis *Isabel Mejia Natividad*[1], Ching-Ho Chang[1], Harmit Malik[1,2] 1) Fred Hutch; 2) Howard Hughes Medical Institute

During spermatogenesis, many animals replace histones with small positively charged proteins that can tightly package DNA, known as sperm nuclear binding proteins (SNBPs). Unlike highly conserved histones, Drosophila SNBPs recently acquired essential functions for fertility and expanded lineage-specific functional paralogs via duplications.To study how SNBPs acquired sperm-specific function in Drosophila, we focus on two young SNBPs, tHMG1 and tHMG2. tHMG1/2 has originated in the Sophorphora subgenus from the duplication of a ubiquitous transcription factor (HmgD or HmgZ) with a high mobility group box (HMGB), and is further tandemly duplicated in D. melanogaster. The roles of tHMG1 and tHMG2 during spermatogenesis are unknown, but they appear to have non redundant functions. Here we show that tHMG1 is under positive selection using population genetic analyses and its knockdowns show a reduction in progeny. However, we did not detect the same signature of positive selection and phenotypes in tHMG2. Consistent with our data, previous studies also show tHMG1 and tHMG2 have different expression and cytological patterns in sperm. We hypothesized that the highly diverged C-terminus, instead of the conserved HMGB domain, between tHMG1 and tHMG2 determine the functional differences between these two recently diverged duplicates. We are generating CRISPR knockouts and transgenic flies of tHMG-1 and tHMG2 to understand their biological function. Our study will highlight how new SNBPs acquired function during sperm development and further shed light on the evolutionary forces shaping SNBPs evolution.

409C Identification of CG4511 as a Novel Regulator of Spermatogenesis *Christopher Petit*, Claire Chaikin, Michaela Marra, Elizabeth Kojak, Stefan Kanzok, Jennifer Jemc Mierisch Loyola University Chicago

Phosducin-like protein 3 (PhLP3) has been shown to possess redox-activity, and it is believed to function as a co-chaperone in the folding of certain cytoskeletal proteins. PhLP3 is very well conserved across the animal kingdom from humans to *Drosophila melanogaster*. PhLP3's homolog in *Drosophila melanogaster* is the uncharacterized CG4511. We find that CG4511 plays a role in the regulation of spermatogenesis in *Drosophila melanogaster.* A P-element inserted in the 5' UTR of *CG4511* leads to a decrease in its expression, infertility in males homozygous for the P-element and testes that fail to produce mature sperm. Further examination of these *CG4511* mutant testes reveals that actin-based individualization cones are absent, as are the needle-like nuclei indicative of mature sperm. Differential interference contrast microscopy and phase contrast microscopy reveal defects in spermatid maturation. Additionally, seminal vesicle size in these *CG4511* mutants is severely reduced. Excision of the P-element restores male fertility, spermatogenesis, and seminal vesicle size. Our results suggest the importance of CG4511 in the regulation of spermatogenesis. Given its hypothesized role as a co-chaperone for the folding of cytoskeletal proteins, CG4511 may be required to promote the folding of cytoskeletal proteins needed for spermatogenesis. The absence of actin individualization cones in males homozygous for the *CG4511* P-element insert supports this hypothesis. We are currently exploring the hypothesis that CG4511 also regulates microtubule dynamics during spermatogenesis.

410A Mutation in *Drosophila Concentrative nucleoside transporter 1* (*cnt1*) alters spermatid maturation *Houda Ouns Maaroufi*[1,2], Lucie Pauchova[1,2], Yu-Hsien Lin[1,2], Lucie Kucerova[1], Ligia Cota Vieirra[1], Lenka Rouhova[1,2], Bulah Wu[1,2], Hanna Sehadova[1,2], Michal Zurovec[1,2] 1) Biology Centre CAS, Institute of Entomology, Czech Republic; 2) Faculty of science, University of South Bohemia, Zoology department, Czech Republic

Nucleoside transporters are essential mediators for nutrient transmission and energy metabolism. Although there have been intensive studies on nucleoside transporters and their biochemical function, no study has investigated the physiological role of concentrative nucleoside transporters (*cnts*). In this study, we use *Drosophila melanogaster* to explore the role of *cnt1*. We generated a mutation in the *cnt1* gene. Our results show partial male sterility. Electron microscopy revealed abnormalities in the sperm tail and mitochondrial defects. In addition, we observed high ATP levels in the male testes. We therefore hypothesise that these phenotypes result in altered sperm motility. These results support the hypothesis that energy balance is required for full maturation of spermatids.

411B Investigation of Y expression in germ cells, if it is modulated by the non-autonomous cues from soma *Sharvani Mahadevaraju*, Brian Oliver National Institute of Diabetes and Digestive and Kidney Diseases, NIH, Bethesda, MD

Drosophila gonads are highly sexually dimorphic with somatic and germ cell components. The karyotype (XX and XY) of somatic and germ cells must match in addition to the germ cell requiring the non-autonomous signals from soma for the production of functional gametes. Y chromosome ~40mb, is mostly heterochromatin contains ~16 known protein coding genes. The Y linked fertility factors are known to transcribe in primary spermatocytes in a specific spatiotemporal manner for ~90 hours during the spermatogenesis that is important for male fertility. Nevertheless, the Y expression if modulated by non-autonomous cues from soma has never been addressed, which is the purpose of this study.
We adapted transcriptomics and generated RNA-seq libraries from gonads of XY larvae (L3) as control and from XY larvae with ectopically expressing *transformer* (*tra*) that will allow us to investigate the Y expression when the somatic sex cue is reversed. The transcriptome analysis revealed that none of the Y linked genes that are expressed in XY gonads (12 protein coding genes, 4 pseudogenes with Transcript Per Million value >1) are expressed when the somatic sex

is reversed with the exception of *flagrante delicto Y* (FDY) that has an autosomal paralog. Our cytological capture of Y transcription (*male fertility factor kl3* (*kl-3*), first exon transcripts detection by *insitu*) supported our transcriptome analysis as *kl-3* is transcribed in XY spermatocytes evidently whereas it was not in XY germ cells when the somatic sex cue is reversed. In case of XXY karyotype, *kl-3* is inactive in germ cells possibly because of female cues coming from the soma. In that case it will be interesting to see if reversing the somatic sex in XXY soma leads to Y transcription that we are currently reviewing.
Our results infer that the non-autonomous male cues from soma (*tra* absence) are necessary for the most Y expression in XY germ cells. The male somatic cues might initiate a special transcriptional program in the germ cells that regulates Y activation which is inhibited when the somatic sex is reversed. Although, it is possible that the male cue from soma is necessary for XY germ cells to progress into the primary spermatocyte stage and proper spermatocyte specific transcription and/or parallel transcriptional program that in turn initiate Y transcription, that we are excited to investigate in near future.

412C Exploring The Sperm Head-Tail Connection Apparatus *Kathleen Mulhern*, Brian Galletta , Nasser Rusan Nation Heart, Lung and Blood Institute, NIH, Bethesda, MD

The Head-Tail Connection Apparatus (HTCA) establishes and maintains the connection between the sperm's nucleus and elongating axoneme. Failure of the HTCA results in decapitation which can lead to male infertility. The structure of the HTCA has been investigated through electron microscopy but only a few proteins have been implicated in establishing and/or maintaining this connection. It is highly likely that there are unknown factors contributing to the integrity of this connection as neither direct nor indirect interactions between the known factors have been elucidated. In order to identify unknown factors, we are conducting a GFP localization screen of proteins in the Drosophila Sperm Proteome (Wasbrough et al 2010). Of the 1108 proteins in the sperm proteome, there are 167 proteins with endogenously-tagged GFP stocks available through Bloomington Drosophila Stock Center. We started this screen by looking at 30 of these 167 available stocks. In this initial selection, we prioritized unnamed proteins and proteins with known cytoskeletal binding activity. So far, we have identified 2 proteins with localizations at or near the HTCA and are following up on both by looking at knockdown phenotypes. We have also identified several proteins which localize to other structures during sperm development. This initial success has encouraged us examine other GFP-tagged lines. While the primary goal of this project is to identify factors involved in the HTCA, our initial data also indicates that this screen will uncover factors involved in other aspects of Drosophila sperm development.

413A The N-end rule Pericentrin degradation is required for centrosome assembly and function in Drosophila spermatogenesis *Ramya Varadarajan*, Brian Galletta, Carey Fagerstrom, Karen Plevock, Nasser Rusan National Heart Lung and Blood Institutes, NIH

Centrosomes are the major microtubule organizing centers (MTOC) that are composed of centrioles and the pericentriolar material (PCM). Centrosomes are essential part of diverse cellular processes that require precise regulation of their protein levels. One protein whose levels must be regulated is Pericentrin – PCNT in humans and PLP in *Drosophila*. Increased PCNT expression and its protein accumulation are linked to many clinical conditions especially in patients diagnosed with cancer, mental disorders, and ciliopathies reflecting the requirement for regulating PCNT level. However, the mechanisms by which the PCNT is regulated remain less explored. In this study, we took advantage of Drosophila spermatogenesis to characterize the mechanistic of PCNT/PLP degradation. Our previous study by Galletta, 2020 demonstrated that PLP levels are sharply downregulated during early spermatogenesis and this regulation is essential to spatially position PLP in the meiotic centrioles. We therefore first performed a structure-function analysis to identify the functional domain required for regulating PLP level and localization. We found that the N-terminal region of PLP is essential to regulate its protein level and depletion of these regions led to PLP stabilization, which in turn mislocalized PLP on the centrioles. As a consequent, the PCM was also mispositioned leading to defects in spermatids, which ultimately compromised the sperm function. Our biochemical and proteomic analysis revealed that the N terminal region of PLP harbors degradation signals and engages interactions with multiple proteosomal components that hinted a precise degradation process to act via N terminal region. To identify the mechanisms, we performed a candidate RNAi screen composed of proteasome components. We found Rad6, UbcD1 and its related UBR box family E3 ligases, Poe (UBR4) and Hyd (UBR5) to promote PLP degradation in spermatocytes and therefore regulate its spatial localization on the centrioles. The identified candidates are known regulators of the N-end rule degradation pathway, in which their substrates are degraded based on the characteristics of the N-termini amino acids that require single or sequential enzymatic mortification to form N-degron. By characterizing the N terminal amino acids, we found that PLP is a potential substrate of the N-end rule pathway. Collectively, our study identified N-end rule pathway to regulate PLP levels for proper centrosome assembly and may also be relevant for PCNT homeostasis in human as well.

414B A mutation in the gene for kinetochore protein Spc25 disrupts both homolog and sister chromatid connections in male meiosis and causes very high levels of meiosis I nondisjunction *Elsie Adams*, Bruce McKee University of Tennessee

Genetic and molecular analyses of ethyl-methanesulfonate (EMS) generated mutants has provided a solid forward genetics approach for discovering novel gene products and their roles in various mechanisms over many decades. Cytological analysis of the 3rd chromosome EMS mutant *122-044* strongly suggests a gene mutated plays role in mediating accurate connections, alignment and segregation patterns of chromosomes in both *Drosophila* male meiosis I and meiosis II. Fluorescence In Situ Hybridization (FISH) analysis with 3rd chromosome specific DNA probe Dodeca reveals 35% of mutant prometaphase I spermatocytes show loss of proper homolog conjunction (n=37), and 83% of mutant anaphase I divisions exhibit homolog nondisjunction of 3rd chromosome autosomes (n=30). Additionally, 20% of metaphase II mutant spermatocytes display premature sister chromatid separation (n=39). Efforts to map this mutated gene led gene candidates within identified a proximal euchromatic region on chromosome arm 3R. Sequencing of genomic DNA from one candidate, the kinetochore gene *spc25*, from *122-044* mutants revealed a potential 5' splice site mutation predicted to generate a truncated Spc25 protein. The combination of our observed meiotic phenotypes appears to be novel in that proper chromosome segregation during meiosis I and II of male *Drosophila* has been understood to rely on two distinct mechanisms - homolog conjunction and sister chromatid cohesion, respectively. However, our understanding of the distinct roles of kinetochore protein complexes during *Drosophila* male meiosis is incomplete. Our study aims to better understand how specifically *spc25* is involved in *Drosophila* male meiotic chromosome segregation as we complete gene specific complementation analyses, but also determine if some phenotypes may be attributed to mutations of additional genes.

415C Robustness of the canonical mitochondrial fusion machinery promotes Nebenkern formation in *Drosophila* spermatids Alina Kolpakova, Shmuel Pietrokovski, *Eli Arama* Weizmann Institute of Science, Rehovot, 76100

Mitochondria, the bioenergetics powerhouses and biosynthetic centers of the cell, are also implicated in many important cellular processes, such as cell death, autophagy, aging, and regulation of immune response and inflammation. Although stereotypically drawn as static organelles, mitochondria are in fact constantly changing shape and subcellular distribution according to function, energy and metabolic demands of the cell. Mitochondrial shapes usually range from small spheres and short tubules to elongated tubules and reticular networks, and these changes are mainly controlled by the balance between two opposing mechanisms of membrane dynamics, fusion and fission (fragmentation). Proper membrane dynamics is essential for maintenance and function of the mitochondria, whereas abrogation of this balance can lead to common diseases, including several neurodegenerative diseases and cancer. The first gene involved in mitochondrial dynamics, *fuzzy onions* (*fzo*), was discovered by Hales and Fuller in 1997 as a mediator of the fusion of the entire *Drosophila* spermatid mitochondria into a giant sphere called Nebenkern. Consequently, orthologs of Fzo, termed mitofusins, have been discovered in organisms from yeast to human, belonging to the dynamin-related protein superfamily of large GTPases. Mitofusins are expressed on the outer mitochondrial membrane (OMM), tethering together adjacent mitochondria by promoting mitochondrial docking through their auto-oligomerization in trans.

In *Drosophila* spermatids, individual mitochondria aggregate near the newly formed haploid nucleus and subsequently coalesce and fuse into a Nebenkern. The Nebenkern is composed of two giant mitochondria wrapped around each other and arranged in an onion-like structure of layers upon layers of mitochondrial membranes. Although detailed ultrastructural description of Nebenkern formation was already reported five decades ago, the molecular mechanisms underlying the formation of this extraordinary organelle remains largely obscure. Furthermore, it remained unknown whether Fzo has been evolved to uniquely promote fusion of the mitochondria into a giant sphere rather than to reticular network, as well as to what extent the canonical fusion machinery might be involved in Nebenkern formation. Here, I will present our recent studies aiming to address these and related questions, presenting our ongoing unpublished work on the molecular and anatomical mechanisms underlying Nebenkern formation.

416A Regulation of *cycB* translation by a four-protein complex in *Drosophila* spermatocytes *Catherine Baker*, Margaret Fuller Stanford Univ Sch 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 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 Fest acts as a scaffold protein, binding Lutin (CG1690) and Syp as well as Rbp4. Lut, like Rbp4, 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 using the heat-shock time-course developed in the lab, where *bam* mutant spermatogonia are subjected to a pulse of wild-type Bam protein under the control of a heat-shock promoter, then differentiate into

spermatocytes and later stages in synchrony. Preliminary data indicate that CycB protein is high by 54 hours post-heat-shock (PHS) in *rbp4*, by 92h PHS in *lut*, and by 104h (but not 100h) PHS in wild-type. This result raises the possibility that Rbp4 and Lut, while both belonging to the same complex, could be repressing *cycB* translation via two distinct mechanisms, perhaps at sequential steps. In contrast, we have found that Syp is required for CycB accumulation in mature spermatocytes. Syp, like Rbp4, binds the 130nt *cycB* spermatocyte 3'UTR. Curiously, Syp binds to Fest and can co-precipitate with both Rbp4 and Lut in the presence but not absence of Fest. Spermatocytes in the *rbp4 syp* double mutant show a transient, early expression of CycB, whereas CycB is never detectable in *lut syp* spermatocytes, suggesting that Syp may act by countering Rbp4 function (but not Lut function). The dynamic off-to-on effect on *cycB* translation is not dictated by any changes in the core interactions within the complex between 72h PHS and 104h PHS.

417B Cellular and molecular basis of transcriptional regulation during spermatogenesis in *Drosophila* *Saurabh Chaudhary*, Sabrina Williams, Shrinivas Dighe, Katia Jindrich, Helen White-Cooper School of Biosciences, Cardiff University, Cardiff, UK

Spermatogenesis provides an excellent model system to study many biological processes, including co- and post-transcriptional gene regulation. The adult *Drosophila* male germline is highly transcriptionally active during the primary spermatocyte stage. The transcriptional complexity in *Drosophila* spermatogenesis is well known, however, the underpinning molecular mechanism regulating the expression of more than 1000 genes, specifically in testes in *Drosophila* remains largely undetermined. Using predominantly *D. melanogaster* as a model, we are employing various state-of-art techniques in the field to understand the cellular and molecular basis of testes specific gene regulation. Testis Meiotic Arrest Complex (tMAC) is required for the activation of transcription of a large number of genes in spermatocytes. This complex comprises several proteins with DNA binding activity and several additional non-DNA binding proteins. We are dissecting how this complex interacts with target promoters by identifying *in vitro* binding motif for each of the DNA-binding domain-containing proteins within tMAC using high throughput SELEX sequencing. For *in vivo* identification of binding sites of each tMAC subunit, we are generating GFP-tagged lines and using high-resolution Chip-Exo, RNA-Seq, and Chip-qPCR in *Drosophila* testes. Testis-specific transcription typically depends on short DNA regions immediately flanking the transcription start sites (TSS). We have generated a set of promoter-reporter constructs and are investigating how these short promoter regions interact with tMAC, and other transcriptional regulatory complexes, to enable gene expression. For some genes, a small number of mutations are sufficient to convert a non-functional promoter into a functional promoter. By integrating the *in vivo* data with the *in vitro* binding site motif information, we will determine which DNA-binding proteins contribute to the binding of tMAC to any specific promoter sequence. This study will further deepen our understanding of how testes specific gene regulation achieves at co- and post-transcriptional levels in *Drosophila*.

418C Characterization of test specific sugar transport and glycolysis genes in *Drosophila melanogaster* *Mark Hiller*, Julia Gazzola, Elizabeth Hughes, Rylee McDonell, Katie Shannon, Emily Pochet, Katlyn Heneghan, Gianna Graziano Goucher College

Sugar is required to provide energy for cellular metabolism and development. The genome of *Drosophila melanogaster* contains twenty-five genes that are annotated as SLC2 type sugar transporters, and five of the sugar transporters appear to be expressed only in the testis. Lactate transport into male germ cells could also be used as a source of ATP, and one gene, *CG12866*, encodes a testis specific Monocarboxylate transporter. Likewise, there are several genes that function in glycolysis that have testis specific homologs or testis specific spice forms. Each of these genes could function during the generation the cellular energy necessary for spermatogenesis or be required for sperm function during fertilization. Mutations in the *sut3*, *sut4*, and *CG14605* putative transporters are fertile. To assess the role of glycolysis and lactate transport, we are using RNAi in the germline and cyst cells of the testis to knockdown function of the lactate transporter and glycolysis genes.

419A Rethinking cyst formation during *Drosophila* spermatogenesis *Rocky Diegmiller*[1], Tomer Stern[1], Yukiko Yamashita[2,3], Stanislav Shvartsman[1,4] 1) Princeton University, Princeton, NJ, USA; 2) Massachusetts Institute of Technology, Cambridge, MA, USA; 3) Whitehead Institute, Cambridge, MA, USA; 4) Flatiron Institute, New York, NY, USA

The process of rapidly and properly producing germ cells is essential for life. In *Drosophila melanogaster* females, germline clusters of 16 cells are formed as the result of four maximally-branched, synchronous divisions from a single germline stem cell. This same pattern of divisions has also been thought to govern *Drosophila* spermatogenesis, where cells in the cyst additionally undergo a round of meiosis to produce 64 identical spermatids. Here, using 3D reconstructions of sturctural elements in developing male germline clusters, we provide evidence to suggest that maximal branching can be lost at the fourth division. We additionally show that the fusome, which allows for intercellular communication and transport within the cyst, is fragmented more frequently in the male germline, suggesting it plays a less vital role than in oogenesis. Overall, this work establishes an important distinction between male and female germline development and introduces future areas of research to investigate the mechanisms governing gametogenesis across species.

420B Using FIB-SEM to create a 3D model of early oogenesis *Stephanie Pellegrino*[1], Irina Kolotuev[2], Lindsay Lewellyn[1] 1) Butler University, Indianapolis, IN; 2) Université de Lausanne, Bâtiment Biophore, Ecublens, Switzerland

The fruit fly serves as an excellent model for studying oogenesis, or female gamete formation. Each egg develops from an egg chamber, which progresses through the stages of oogenesis as part of a developmental array known as an ovariole. At the anterior of each ovariole lies the germarium, which contains the germline and somatic stem cells which will divide to give rise to each newly formed egg chamber. Much has already been learned about oogenesis using a combination of fluorescence and electron microscopy to study specific structures and proteins; however, a complete picture of the organization of germline and somatic cells and their intracellular structures and organelles is lacking. To learn more about the early stages of oogenesis, we are rendering a large data set collected using Focused Ion Beam Scanning Electron Microscopy (FIB-SEM) to generate a complete 3D model of the germarium and an early-stage egg chamber. Our lab is primarily interested in the structure of the germline ring canals, or intercellular bridges, which allow nurse cells to transfer their cytoplasmic contents into the growing oocyte. Electron microscopy has previously revealed the presence of extensive membrane protrusions, or microvilli, surrounding the germline ring canals. This microvilli meshwork is thought to maintain the anchoring and stability of the ring canals during egg chamber growth, yet the stage of oogenesis when these protrusions first appear and their spatial distribution within the germline is not known. Therefore, we hope to characterize changes in the size and structure of the germline ring canals and the organization of germ cell membranes during early oogenesis. Once completed, we envision that this dataset can become a valuable resource that can be further mined by other researchers in the field.

421C The neurodegeneration gene *iPLA2-VIA* is required for mitochondrial maintenance in the *Drosophila melanogaster* female germline, with autonomous and non-autonomous components *Tamar Soussana*, Eliezer Heller, Sarah Liberow, Surya Banerjee, Adina Schonbrun, Sogol Eizadshenass, Matthew Lubin, Josefa Steinhauer Yeshiva University

Neurodegenerative disease (ND) is a major health issue world-wide, yet the underlying mechanisms are still ill-defined. Loss of function mutations in the gene *PLA2G6*, encoding the group 6A calcium-independent phospholipase A_2, iPLA2-VIA, are associated with severe ND in humans, including autosomal recessive dystonia-parkinsonism. The orthologous *Drosophila melanogaster* gene encodes a highly similar protein to the human gene product, 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 nurse cells. Furthermore, a null mutation in *iPLA2-VIA*, generated in our lab, causes age-related 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 technique 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 strong non-autonomous component to the germline mitochondrial defects. We currently are knocking down *iPLA2-VIA* in select somatic tissues, including neurons, muscle, fat body, and ovarian follicle cells, to determine which specific tissues contribute to the germ cell phenotype. In complementary studies, we are conducting tissue-specific rescue experiments using a wild-type cDNA transgene or a catalytically-inactive cDNA transgene, both of which rescue age-dependent locomotor decline. Finally, we have shown that *iPLA2-VIA* does not interact genetically with *pink1*, another neurodegeneration gene important for germline mitochondrial integrity, suggesting parallel pathways. We expect our findings to have relevance to the mechanisms underlying PLA2-associated neurodegeneration.

422A Spargel/dPGC-1 is a closer ancestor to mammalian PRC-1 with an RRM domain that is functionally essential for oogenesis *Swagota Roy*, Mohammed Shah Jalal, Sabarish Nagarajan, Atanu Duttaroy The Howard University, Washington DC

The transcriptional coactivators Peroxisome Proliferator Activated Receptor-1 (PGC-1) play a key role in wide range of physiological processes in mammals including mitochondrial biogenesis, oxidative metabolism, and adaptive thermogenesis. There are three mammalian PGC-1 paralogs: PGC-1α, PGC-1β, and PRC-1. Spargel/dPGC-1 is an ancestral PGC-1 homolog in Drosophila that is predominantly expressed in the ovaries during adult life. Ovary-specific knockdown of *srl* induces complete sterility indicating that Spargel plays an essential role in oogenesis and ovarian growth. Spargel shares many structural features with PGC-1 proteins including the Serine-Arginine rich repeats (RS), RNA recognition motif (RRM) and a nuclear localization signal (NLS). Due to their functional redundancy, attempts to make domain specific deletions in PGC-1 failed to demonstrate the role of RRM in mammalian system. We generated two *srl* mutant lines using CRISPR/CAS9 system: (1) A 3336 bp deletion was created within the *srl* beginning from Exon 2 (srl^{del}), and (2) RRM domain specific deletion (ΔRRM). Homozygous srl^{del} embryos complete the embryonic development but can't

proceed beyond 1st instar larval stage as these larvae failed to emerge out of the chorion. This suggests that Spargel function is not limited to oogenesis, but it is also essential for postzygotic development. Mitotic clones of *srl*^{del}established it as an amorphic allele which may be the reason why most *srl*$^{del/del}$ egg chambers don't survive beyond previtellogenic stages. Incidentally, germ line homozygous knockouts of PGC-1α or PGC-β are viable and fertile with no global changes in mitochondrial number or morphology, however a germ line knock-out of the *PPRC1* gene in mice results in neonatal lethality. Phylogenetic analysis supports that Spargel is actually a closer ancestor to mammalian PRC-1 as compared to PGC-1α or PGC-β. Homozygous *srlΔRRM* females are viable but carries very few mature eggs in their ovaries. Further analysis shows the infertility of *srlΔRRM*females stems from disrupted actin cable and cortical actin loss in nurse cells resulting in dumpless phenotype. Together, this collection of *spargel* mutants is helping us to address (1) the role of Spargel on ovarian growth and development, and (2) to determine the role of Spargel in postzygotic development.

423B Exploring the role of Oatp74D, an Ecdysone Importer, in the *Drosophila* ovary. *Amanda Powell*, Elizabeth Ables East Carolina University

Oogenesis is the progression of germ cells through mitotic expansion, differentiation into an oocyte, and successful completion of meiosis. Steroid hormones play critical roles in this process in diverse organisms. In *Drosophila*, the main steroid hormone, ecdysone, facilitates female fertility in part by promoting germline stem cell self-renewal. Foundational studies demonstrated that ecdysone is synthesized primarily in egg chambers during mid-oogenesis; however, more recent data suggests that somatic escort cells, which support germline stem cell differentiation, may also produce ecdysone. Understanding how ecdysone is transported and received in the ovary would help resolve these disparate results. Recent studies by the Yamanaka lab support the hypothesis that ecdysone needs Oatp74D, a membrane transport protein, to import ecdysone into cells, challenging the popular assumption that steroids passively transport through membranes. Oatp74D is well-characterized in the *Drosophila* blood brain barrier but may have roles in steroid hormone uptake in other cells. Here, we explore whether Oatp74D is necessary for ecdysone-mediated processes during oogenesis. We use transgenic and immunofluorescence approaches to examine localization of Oatp74D in the ovary. We then test whether Oatp74D knockdown in escort cells effects oogenesis and fecundity. Our preliminary results suggest that Oatp74D is not necessary in escort cells for maintenance of germline stem cells but may promote germ cell differentiation. This data will further our understanding of how ecdysone signaling regulates optimal oocyte production.

424C Regulation of Delta-Notch pathway by mitochondrial signaling during *drosophila* oogenesis *Yipeng Du*, Matthew Sieber UT Southwestern Medical Center

Development in all organisms requires the coordination of cellular metabolism with the proper regulation of signaling pathways. For example, changes in mitochondria metabolism have been associated with cell specification and differentiation in many developmental systems. However, despite this association, very little is known about how mitochondrial metabolism regulates developmental pathways. Our lab utilizes *Drosophila*, mammalian cells, and mice to examine the conserved metabolic mechanisms that drive development and disease progression.
To understand the role for mitochondria in developmental signaling, we conducted a genetic screen of mitochondrial proteins. Using this approach, we discovered that a specific subset of mitochondrial genes, when disrupted, cause defects in development of the *Drosophila* ovary and intestine. In particular, we found that inhibition of *prohibitin (PHB in mammals)* in germ cells caused a block in follicle cell differentiation. Loss of *prohibitin* in the mitochondria causes: a reduction of membrane potential, decreased ATP production, and increased cellular ROS. These defects in mitochondrial oxidative metabolism cause an increase in follicle cell number, reduced size of follicle cell nuclei, and defects in follicle cell differentiation in *prohibitin* RNAi egg chambers. When we examine the localization of the Notch ligand, Delta, we find that it accumulates in large puncta in *prohibitin* RNAi egg chambers and fails to reach the plasma membrane. Interestingly, lowering ROS by overexpression Catalase and Sod2 genes partially rescue the Delta aggregation developmental phenotypes in ovaries. In addition, we have found that overexpression of Rab11 which is a key protein of recycling vesicles rescue the phenotype of Delta aggregation in *prohibitin* RNAi egg chambers. These data suggest that mitochondrial ROS regulates Delta trafficking in Rab11 dependent manner. Overall, our work provides intriguing new evidence that identifies the regulation of receptor trafficking as a novel mechanism for how mitochondria regulate differentiation. Moreover, this work provides a foundation for future studies of how changes in mitochondria metabolism promotes cancer progression by regulating Notch signaling.

425A A Cytological F1 RNAi Screen for Defects in *Drosophila melanogaster* Female Meiosis *William Gilliland*, Amanda Bowen , Kelly Conger, Doreen Elrad, Marcin Marciniak, Denny May, Gabrielle Presbitero DePaul University

Forward genetic screens induce mutations, make the mutated chromosomes homozygous, and then homozygotes for the phenotype of interest. When studying female meiosis, the phenotype is usually nondisjunction from chromosome segregation errors. This means that mutant females must be viable and fertile, and any meiotic genes that are lethal or sterile when homozygous cannot be recovered by this approach. Our lab has screened the VALIUM22 collection produced by the Harvard TRiP Project, which contains RNAi constructs targeting genes known to be expressed in the germline in a vector optimized for germline expression. By driving RNAi with GAL4 under control of a germline-specific

promoter (*nos* or *mat-alpha)*, we can test genes that would be lethal if knocked down in all cells, and by examining unfertilized metaphase-arrested mature oocytes, we can identify defects associated with genes whose knockdown results in sterility.
We screened this collection to identify genes that disrupt either of two phenotypes: the ability of meiotic chromosomes to congress to a single mass at the end of prometaphase, and the sequestration of Mps1-GFP to unknown structural filaments in response to hypoxia. After screening the ~1500 lines in the collection, we obtained multiple hits for both phenotypes, identified novel meiotic phenotypes for genes that had been previously characterized in other tissues, and found novel phenotypes for several previously uncharacterized genes.

426B Structural changes in centrosomes correlate with activation of a checkpoint that triggers germline stem cell loss *Isabella Perales*[1], Tingting Duan[1], Pamela Geyer[1,2] 1) University of Iowa; 2) NIH

Homeostasis of Drosophila germline stem cells (GSCs) depends upon the integrity of the nuclear lamina (NL). Indeed, loss of the NL protein emerin blocks germ cell differentiation and causes GSC death due to activation of two DNA damage response kinases ATM- and Rad3-related (ATR or Mei-41) and Checkpoint kinase 2 (Chk2 or Loki). Previous studies suggested that checkpoint activation in *emerin* mutants results from a thickening and lobulation of the NL, due to insertion of enlarged interphase centrosomes that carry excess pericentriolar material (PCM) to nucleate microtubules. To understand whether NL distortion or centrosome structure is the primary activator of the ATR/Chk2 checkpoint, we used the Gal4-UAS system to overexpress two NL proteins, the inner nuclear membrane protein Kugelkern (Kuk) and the A-type lamin (Lamin C), chosen based on previous findings that their increased accumulation caused NL distortion. Although over-production of both proteins was achieved, only Kuk caused NL deformation in GSCs, demonstrating that Lamin C over-expression has cell type specific effects on NL structure. Whereas Kuk overexpression caused NL distortion, interphase centrosome structure was unaffected, with centrosomes localizing outside of the nuclear envelope and retaining low levels of PCM. Notably upon Kuk overexpression, GSCs were maintained and oogenesis was sustained. Based on these findings, we conclude that NL distortion alone is not sufficient for activation of the ATR/Chk2 checkpoint. Instead, our findings suggest centrosome structural changes might drive checkpoint activation in GSCs.

427C Stonewall promotes germ cell to oocyte transition by promoting heterochromatin maintenance during Drosophila oogenesis *Noor Kotb*[1,2], Ankita Chavan[3], Lydia Proskauer[4], Elliot Martin[2], Madhav Jagannathan[3], Prashanth Rangan[2] 1) Department of Biomedical Sciences/ Wadsworth Center, University of Albany, Albany, NY; 2) Department of Biology/RNA institute, University of Albany, Albany, NY; 3) Department of Biology, Institute of Biochemistry, ,ETH Zürich, Zurich; 4) Colgate University, Hamilton, NY

During oogenesis, germ cells differentiate into an oocyte, which upon fertilization gives rise to a totipotent zygote. In *Drosophila*, the transition from germ cell to an oocyte requires SETDB1 dependent heterochromatin formation to silence germ cell differentiation genes after the oocyte specification. These silenced germ cell genes are often interspersed between euchromatic genes that are actively transcribed to promote oocyte fate. As heterochromatin is prone to spreading to euchromatic regions, how such heterochromatic territories remain confined during oogenesis remains poorly understood. Here, we find that the DNA binding protein Stonewall (*Stwl*), is required to establish a boundary between heterochromatic and euchromatic domains. We find that *stwl* binds to promoter regions of the silenced germ cell differentiation genes and promotes the maintenance of heterochromatin at these loci. Loss of *stwl* results in loss of heterochromatin spreading, dysregulation of transcription and loss of oocyte fate resulting in mid-oogenesis death. Thus, during germ cell to maternal transition, *Stw*l acts a boundary element to maintain heterochromatin to promote an oocyte fate.

428A *fs(1)K741* is a female sterile allele of the gene *Sxl* and disrupts *Sxl* splicing *Jillian Gomez*[1,2], Myles Hammond[2,3], Stephen Kucera[2], Brian Oliver[1], Leif Benner[1,4] 1) National Institute of Diabetes and Digestive and Kidney Diseases, NIH, Bethesda, MD; 2) Department of Biology, University of Tampa, Tampa, FL; 3) Integrative Genetics and Genomics, University of California-Davis, Davis, CA; 4) Department of Biology, Johns Hopkins University, Baltimore, MD

Sxl is the master sex-determination gene and acts via a sex-specific splicing mechanism. The locus has often been discussed as two simple steps in females: the production of an early protein involved in establishing autoregulatory splicing and the production of a late protein maintaining the female-specific splice. We mapped a female sterile allele, *fs(1)K741*, to the *Sxl* locus through deficiency mapping and duplication rescue. We used whole genome sequencing to determine *fs(1)K741* to be a single point mutation (C>T) in the male-specific exon 3 of *Sxl*. CRISPR-Cas9 and a ssOligo donor directed to the point mutation in *fs(1)K741* were employed to recreate the mutation in wild-type females. This point mutation failed to complement *fs(1)K741*, confirming it to be the cause of sterility and that *fs(1)K741* is an allele of *Sxl*, thus called $Sxl^{K741\text{-}1}$. RT-PCR of $Sxl^{K741\text{-}1}$ ovaries showed both male and female-specific *Sxl* splicing products, while RT-PCR on $Sxl^{K741\text{-}1}$ carcasses showed only female-specific *Sxl* splicing. This indicates that aberrant splicing is restricted to the ovary. We wanted to determine how this mutation in the male exon was disrupting *Sxl* splicing. Homozygous $Sxl^{K741\text{-}1}$ females have a temperature-sensitive sterility and lethality effect. At 18°C, homozygous females are 28% viable and 80%

fertile. At 29°C, homozygous females are 37% viable and completely sterile. Two duplications, *Dp(1;3)DC489* and *Dp(1;3)DC490*, rescued viability of $Sxl^{K741\text{-}1}$ females. However, only *Dp(1;3)DC490* rescued fertility. The difference between the two duplications is that *Dp(1;3)DC490* contains the full *Sxl* reading frame while *Dp(1;3)DC489* only contains the early *Sxl* reading frame, possibly indicating that the viability defect in $Sxl^{K741\text{-}1}$ is due to aberrant early protein function. We created loss-of-function frameshift mutations specific to the early, late, and all proteins denoted as, Sxl^{Early}, Sxl^{Late}, and Sxl^{All}, respectively. Sxl^{All} failed to complement viability of $Sxl^{K741\text{-}1}$ at both 18°C and 29°C. Sxl^{Late} failed to complement viability at 18°C, but was subviable and subfertile, at 29°C. Sxl^{Early} complemented viability and fertility of $Sxl^{K741\text{-}1}$ at both 18°C and 29°C. These results are somewhat at odds with the duplication results since the duplication only containing the early protein rescued viability. Still, it is clear from these new mutants that $Sxl^{K741\text{-}1}$ is not deficient in the establishment step of *Sxl* splicing, and the mutation is likely disrupting the maintenance of *Sxl* autoregulation. Although *Sxl* has previously been thought of and described as a simple step-wise autoregulatory cascade initiated by the early protein and maintained by the late protein, $Sxl^{K741\text{-}1}$ clarifies that the autoregulatory process of *Sxl* is much more complex. Understanding how this mutation in the male exon of *Sxl* leads to subviability and sterility will help researchers further understand the complex genetics of *Sxl*.

429B The expression of OVO isoforms throughout *Drosophila* development *Savannah Muron*[1,2], Leif Benner[1,3], Brian Oliver[1] 1) National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD; 2) Department of Biology, University of Tampa, Tampa, FL; 3) Department of Biology, Johns Hopkins University, Baltimore, MD

An important component in the continuity of life is sexual reproduction, yet the genetic determinants of germline differentiation to create a sex-specfic gamete remain largely uncovered. Exploring the activity of transcription factors essential to these processes give needed insight to this concept. In *Drosophila,* OVO is required for female germ cell viability. *ovo* is a self-regulating gene that encodes two antagonistic isoforms, OVO-A and OVO-B, with OVO-A acting as a repressor and OVO-B acting as an activator. To reveal the behavior of OVO, we chose to visualize its activation during germline development and oogenesis. A sequence containing a 3x-FLAG-HA tag was placed at 4 locations in the open reading frame of the *ovo* locus, allowing us to tag different isoforms and visualize its expression and cellular localization. This tag was placed at the N-terminus of the OVO-B transcript ($ovo^{ovo\text{-}Nterm}$, tagging both OVO isoforms), the N-terminus of the OVO-A transcript ($ovo^{ovo\text{-}A}$, tagging OVO-A), the C-terminus of all transcripts ($ovo^{ovo\text{-}Cterm}$, tagging both OVO isoforms), and the N-terminus of an OVO-B transcript in which the promoter of OVO-A was deleted ($ovo^{ovo\text{-}\Delta AP}$, tagging OVO-B). Staining in adult ovaries of these different alleles revealed the presence of nuclear OVO in the germarium and differentiating germ cells of the adult ovaries. $ovo^{ovo\text{-}Cterm}$ had a persistent staining pattern from germline stem cells throughout developing egg chambers, while $ovo^{ovo\text{-}Nterm}$ showed a weaker staining intensity in the germline stem cells but stronger in region 2a of the germarium and stage 4 egg chambers. $ovo^{ovo\text{-}\Delta AP}$ replicated staining patterns of $ovo^{ovo\text{-}Nterm}$. $ovo^{ovo\text{-}A}$ appears to have a decreased staining intensity than the other alleles but follow the same staining pattern as $ovo^{ovo\text{-}Nterm}$. We also looked at the expression of OVO in the adult male germline and were able to detect the presence of OVO in $ovo^{ovo\text{-}Nterm}$ and $ovo^{ovo\text{-}Cterm}$ in germ cells near the hub, but staining was lost in differentiating spermatogonia, while $ovo^{ovo\text{-}A}$ staining was not detectable. Immunoblotting analysis of the alleles show that $ovo^{ovo\text{-}Nterm}$ and $ovo^{ovo\text{-}\Delta AP}$ have identical banding patterns, but the $ovo^{ovo\text{-}Cterm}$ had different protein bands, while $ovo^{ovo\text{-}A}$ protein was not detectable. The localization and banding patterns between $ovo^{ovo\text{-}Cterm}$ and $ovo^{ovo\text{-}Nterm}$ suggest that OVO may be regulated at the protein level. We are continuing to determine the expression pattern of these alleles during embryogenesis and larval development in males and females to gain a better insight into *ovo* expression and regulation.

430C Analysis of RNA Helicase Me31B's Molecular Mechanism in Germline Development by Motif Mutations Carol Dilts, Aidan McCambridge, John Eshak, Brooke Pumnea, Yousif Mukatash, Joseph Jansky, Noor Malik, *Ming Gao* Indiana University Northwest

Drosophila Me31B (DDX6) is a conserved ATP-dependent RNA helicase that plays role in post-transcriptional RNA regulation to ensure the correct spatial and temporal expression of germline mRNAs. This, in turn, is crucial for the proper germline development in many animals. However, Me31B's role and molecular mechanism are not clear. To study this, we aim to first understand the functions of important domains/motifs of the protein. Therefore, we used CRISPR gene-editing technique and generated *Drosophila* strains mutant for functionally important motifs in Me31B including the two RecA-like domains, N-terminal domain and C-terminal domain, motifs involved in ATPase and helicase activities such as DEAD-box motif and HRIGR motif, motifs that cause human developmental defects if mutated such as QxxR motif, and protein-binding motifs such as FDF-pocket motif and W-pocket motif. Further, we are conducting a series of phenotype characterization experiments on the mutants including quantification of the expressed mutant proteins, confocal microscopic examination of the mutant proteins' localization in the ovaries, and fertility assays of the mutant strains. Considering that Me31B/DDX6 family proteins are also expressed in certain soma like neurons and other organisms such as yeast, worm, mouse, and human, the results from this study could further help us understand Me31B/DDX6 family proteins' likely conserved roles in different cell types and species.

431A Size Regulation within the Germline of the Developing Egg Chamber. *Zoe Herdman*, Umayr Shaikh, Lindsay

Lewellyn Butler University

The relationship between the size of a cell and the size of its intracellular structures must be precisely regulated for proper development and functioning of the mature organism; however, the mechanisms that establish and maintain this relationship are not known. Much of the work on organelle size scaling has focused on individual cells or organelles, with some looking at how size scaling is maintained during development. However, it is not known whether the size of multiple organelles is coordinately regulated, and how cell size and organelle size are regulated within a syncytium or group of connected cells. Because many cell types exist within a syncytium, understanding size scaling in this context is important. We have used the developing fruit fly egg chamber as a model system to study the size relationship between multiple structures within the germline syncytium throughout oogenesis. The developing egg chamber contains a cluster of 16 germ cells connected by intercellular bridges, or ring canals. It has been observed that the size of the germline ring canals exhibits spatial variation with the smallest ring canals at the anterior of the egg chamber and the largest ring canals at the posterior near the oocyte. Recent work has also confirmed that nurse cells and their nuclei show a similar size distribution within the germline. Therefore, it is interesting to consider whether the size of these structures may be coordinately regulated, and whether altering the size of one structure (either throughout the germline or clonally) would impact the size of the other structure. We have used a combination of RNAi (Pendulin, Dacapo, or Ctf4), overexpression (Pendulin) and mosaic analysis (*myc, arpC1, dock*) to try to alter either nuclear size or ring canal size in some or all cells within the germline. We used Fiji to measure the diameter of the ring canals, the size of the nurse cell nuclei (which has been used as a proxy for cell size), and the overall dimensions of the egg chamber. Preliminary data suggest that altering nuclear size within the entire germline does impact ring canal size, and that in mosaic tissue, there is typically a correlation between cell size, nuclear size, and ring canal size. In the future, we hope to further explore whether the size of these structures is coordinately regulated, and if so, to identify the underlying mechanisms that are involved.

432B Nuclear and ring canal growth in the germline of the developing egg chamber *Kathleen Sherlock*, Julia Wilson, Umayr Shaikh, Daniel Adan, Lindsay Lewellyn Butler University

The size of intracellular structures must be tightly controlled in order to maintain normal cell function. This is especially important when cells are part of growing tissues or organs. However, despite its importance, few studies have analyzed how the size of organelles or other structures changes during development. The developing egg chamber provides a unique model in which to study the relative growth rates of multiple structures. The egg chamber is composed of a cluster of germ cells (an oocyte and 15 supporting nurse cells) surrounded by a layer of somatic epithelial cells. In addition to the growth of the overall egg chamber, it is also known that multiple structures within the egg chamber grow as well. For example, the nurse cells, their nuclei, and nucleoli increase in size as the egg chamber develops, and the intercellular bridges, or ring canals, that connect the germ cells also significantly increase in diameter. Here, we are able to take advantage of differences in egg size both within *D. melanogaster* and between *Drosophila* species (*D. pseudoobscura, D. melanogaster, D. santomea, D. yakuba, and D. virilis*) to test whether the relative rates of growth of the germline ring canals and nurse cell nuclei are consistent in developing egg chambers of different sizes. To determine whether these growth rates are impacted by the location of the structure within the egg chamber, we specifically focus on two sets of nurse cells and their associated structures – the nurse cells directly connected to the oocyte and those located at the anterior of the egg chamber. In addition, we test whether altering oocyte growth impacts the growth of the germline ring canals or nurse cell nuclei. In the future, we hope to determine how these scaling relationships are maintained during dramatic tissue growth and to test whether the same scaling relationships are observed in more distantly related species.

433C Mob family proteins and Tricornered kinase are required to form dorsal appendages of the *Drosophila* eggshell *Keala Watson*[1], Juan Carlos Duhart[2], Laurel Raftery[1] 1) School of Life Sciences, University of Nevada, Las Vegas, Las Vegas, NV; 2) Current Address: Dept. of Neuroscience, Jefferson University, Philadelphia, PA

Formation of complex tissue structures involves multiple signaling pathways to coordinate cells. In *Drosophila*, epithelial cells form tubular structures to shape the dorsal appendages on the eggshells. We found that components of a Hippo-like intracellular signaling pathway are involved in shaping dorsal appendages, in particular, the core NDR kinase tricornered (Trc) and several Mob proteins. In humans, mice and flies, Mob proteins bind to NDR kinases and increase their activity. There are 4 Mob genes in *Drosophila*: *mats* (encoding Mob1), *mob2*, *mob3*, and mob4. We made a *mob2* knockout allele by CRISPR/Cas9-aided homologous recombination, and found that homozygous females lay eggs with variably shorter dorsal appendages than control females. Knockdown of Tricornered kinase throughout the follicle cells was predominantly associated with defects in tube formation, with a minority of eggshells showing short dorsal appendages. These data suggest that another Mob protein associates with Tricornered for early dorsal appendage tube formation. We are testing the phenotypes of follicle cell knockdown for each of the other three Mob proteins, to identify a candidate Mob partner for Tricornered function in early dorsal appendage tube formation.

434A Physiological and functional implications of differentially enriched transcripts on eRpL22-family polysomes *Caroline Pritchard*, Vassie Ware Lehigh University, Bethlehem, PA

The *Drosophila melanogaster* eRpL22 ribosomal protein 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 endocytosis, autophagy, and mTOR signaling. Some pathways were over-represented in both polysome types, but these were derived from unique transcripts.

Transcripts of genes functionally implicated in human disease, given by Human Phenotype Ontology (HPO) term association, were over-represented on eRpL22-like polysomes (961:496 terms). 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 (181:8 terms). We have previously shown differential expression of eRpL22-like protein and specific knock-down phenotypes in several analogous regions in the fly.

Conditional knock-out of eRpL22-like resulted in many morphological defects within the ovary, including disruption of the germline stem cell niche, ectopic rounded follicular epithelium cells, oocytes with dual (bifurcated) nuclei, double-anteriorized eggs, and specific spatiotemporal patterns of cell death & oogenesis arrest. This constellation of phenotypes suggests eRpL22-like has a role in ensuring proper cell polarity in early development and the ovary is a viable system to investigate the cellular and molecular basis of these defects.

Taken together, these data broaden the context for essential roles of eRpL22-like across multiple developmental processes.

435B ***Identification of E2 ubiquitin-conjugating enzymes required in Drosophila male meiosis*** *Andrea Binder*, John Tomkiel Dean University of North Carolina at Greensboro

Ubiquitination is a post-translational modification in which a small protein called Ubiquitin is covalently attached to Lysine residues of target proteins to specify their degradation, alter their activity or localization. In a multistep process, an E1 ubiquitin ligase activates Ubiquitin and transfers it to an E2 ligase. The activated E2 then interacts with an E3 ligase which specifies the target. In *D. melanogaster* there is a single E1 ligase (Uba1), 29 predicted E2 ligases and 156 predicted E3 ligases, allowing for a multitude of potential ubiquitin ligase complexes. This suggests that this complexity is needed for temporal or tissue-specific ubiquitination, however relatively few complexes and their targets have been defined. Here we sought to identify which E2 ligases are required in the male germ line. We used four *GAL4* drivers (*bam,* C135*, nanos*, T110) in combination with UAS-RNAi constructs to knock down expression of all 29 E2 ligases and Uba1 in the male germline. Test males were assayed for fertility, were examined cytologically for visible defects in spermatogenesis and monitored genetically for fourth chromosome missegregation. Knockdowns of both *taf1* and *ubc6* produced the most severe phenotype of male sterility resulting from failure to enter meiosis. This phenotype was also observed for the knockdown of *uba1*. Meiotic chromosome segregation defects were observed in the knockdowns of six E2 ligases (*bruce, CG7656, CG8188, ubc2, ubc10* and *ubcE2H*). Fourth chromosome missegregation (which may not be detectable cytologically) was observed via genetic crosses for knockdowns of four additional E2 ligases (*CG4443, CG9602, CG10862 and CG17030*). Our results reveal that multiple ubiquitin ligase complexes are necessary for entry into meiosis and meiotic chromosome segregation in males. Several of these have not formerly been implicated in meiosis and future work towards understanding their roles will be focused on identification of their E3 ligase partners and their protein targets.

436C A Borealin-HP1 Interaction Regulates Chromosome Passenger Complex Binding to Chromosomes and Movement to Microtubules *Manisha Persaud*, Kim McKim, Janet Jang Rutgers University -- New Brunswick

Mitosis and meiosis are the most exciting and elaborate processes that occur during the life of dividing cells however, defects in these processes can lead to aneuploidy and lethal phenotypes. Errors in chromosomal segregation in oocytes lead to infertility and birth defects. We use Drosophila to understand the mechanisms of homologous chromosome bi-orientation that occurs during meiosis I, and the features of the oocyte-spindle that make it susceptible to segregation errors. The chromosome passenger complex (CPC) is a highly conserved master regulator that is required for oocyte spindle assembly, kinetochore assembly and homologous chromosome biorientation. It is a complex consisting of the proteins INCENP, Aurora B kinase, Survivin and

Borealin. The CPC is recruited by the chromosomes and then moves to the microtubules. The mechanisms of this movement and the CPC signaling pathway that recruits and promotes the activity of kinetochore and spindle proteins remains largely unknown. Our previous research has led to a model that Borealin promotes spindle assembly in Drosophila oocytes by interacting with nucleosomes or the chromatin protein HP1. It was hypothesized that a Borealin-HP1 interaction is necessary for CPC binding to the chromosomes and movement to the microtubules. We are testing this model by mutating two domains within Borealin that may support this function: (1) a domain for binding HP1 and (2) a domain for binding microtubules. For these experiments, we have generated two Borealin shRNA lines and found that depletion of Borealin by RNAi showed phenotypes similar to aurB and or Incenp RNAi; complete loss of kinetochore and spindle assembly. Interestingly, many of the oocytes exhibited fragmented karyosomes, suggesting a role for the CPC in oocyte chromatin organization. We are currently generating RNAi resistant transgenes to that express borr mutants that are defective interacting with either nucleosomes / HP1 or microtubules. Parallel to these studies, we are examining the phenotype of Incenp mutants predicted to be defective in microtubule interactions and trying to directly detect interactions between HP1 and either INCENP or Borealin in oocytes.

437A Regulation of Meiotic Kinetochore-Microtubule Attachments by the RZZ Complex *Joanatta Shapiro*, Janet Jang, Kim McKim Waksman Institute of Microbiology, Rutgers University, Piscataway, NJ

Meiosis is a conserved process of cell division occurring in sexually reproducing eukaryotes which produces gametes with half the number of chromosomes. Errors in this process result in infertility, miscarriages, and genetic disorders. One poorly understood protein in meiosis, ROD, is a component of the ROD-ZW10-Zwilch (RZZ) complex and localizes to the kinetochores. The RZZ complex is involved in attachment error correction and the spindle assembly checkpoint (SAC) during mitosis. Error correction involves the selective stabilization and destabilization of kinetochore-microtubule (KT-MT) attachments while the SAC delays entry into anaphase until accurate attachments form. Mitotic evidence suggests that RZZ at the kinetochores prevents the formation of premature stable attachments. Attachment stabilization requires RZZ removal, a process called streaming which is suggested to depend on the microtubule motor protein Dynein and its recruitment factor Spindly. However, the role of ROD in these processes is not well characterized in female meiosis, which is unique in that it lacks the centrosomes and involves the segregation of homologous chromosomes rather than sister chromatids.

To analyze the function of ROD in female meiosis I, we observed ROD behavior in mutant or RNAi oocytes for proteins hypothesized to interact with RZZ. Female Drosophila depleted of ROD in the ovaries are sterile and have chromosome orientation defects, suggesting that ROD is required for correct KT-MT attachments. We found that ROD recruitment to the kinetochores is mediated by amino acids 154-840 of the kinetochore protein SPC105R. Furthermore, while ROD is not required for the creation of the initial KT-MT attachments, it may have a role in preventing or correcting erroneous attachments. Surprisingly, ROD removal may not be required for creating stable attachments, as in mitosis. Therefore, meiotic RZZ streaming may depend on the cell cycle stage rather than attachment status. We also found that ROD streaming in oocytes is at least partly Spindly-independent even though Spindly is required for meiosis I, suggesting potential Dynein-independent mechanisms of RZZ removal in meiosis. In the absence of microtubules, ROD expands around the kinetochores, likely to facilitate microtubule capture and contribute to SAC signaling. Determining how ROD regulates microtubule attachments will provide valuable insight into how pairs of homologous chromosomes segregate to maintain meiotic fidelity.

438B Genome-wide RNAi screen for new meiotic genes in Drosophila melanogaster *Joel Sop*, Tyler DeFosse, Ayla Boyd, Faith Verderose, Joanatta Shapiro Rutgers University

The sex cells, egg and sperm, are formed through a form of cellular division known as meiosis, resulting in four genetically unique daughter cells. During meiosis, accurate chromosome segregation is vital for reproductive success. Occasionally, chromosomal segregation errors occur, resulting in gametes with an incorrect number of chromosomes, a condition known as aneuploidy. Aneuploidy is known to be the leading cause of infertility in women. Aneuploids that survive cell division can cause spontaneous abortions or genetic disorders including Down's Syndrome. Aneuploidy in women rapidly rises after the age of 35, a phenomenon known as the Maternal Age Effect. While several genes required for meiosis have been identified in *Drosophila*, most of these were identified in limited forward genetic screens or based on conservation in other organisms. Many meiotic genes remain to be identified, particularly those required for fertility or if they are poorly conserved. The goal of this project was to identify and characterize new meiotic genes in the *Drosophila melanogaster* genome. Using tissue expression patterns from the FlyAtlas and modENCODE databases, we identified 542 *Drosophila* genes of interest because their expression levels are elevated in meiotically active tissues. RNAi approaches were then used to make a germline targeted knockdown with the UAS/ Gal4 system to observe which of the genes of interest caused significant errors in meiosis (aneuploidy) or fertility. Out of the 265 genes we have tested

so far, 67 were identified due to increased nondisjunction or sterility. We are currently prioritizing these genes and selecting a subset for further study.

439C Characterization of the Immune Deficiency Pathway during female meiosis in *Drosophila melanogaster* *Sarah Mashburn*, William Gilliland DePaul University, Chicago IL

Organisms can defend against pathogens by significantly increasing the diversity of their progeny, so that some progeny are more likely to survive infection. This leads to the prediction that infection should cause an increase in recombination rates. This prediction was confirmed in a published study where female *Drosophila melanogaster* that were infected with the gram-negative bacteria *Providencia rettgeri* had increased recombination rates compared to control flies. However, the mechanism of this rate modulation is unknown. Our lab conducted an RNAi screen to identify genes that cause defects during chromosome congression, which identified *mustard (mtd)* as causing ~40% congression failure. We show this is caused by a 95% reduction in recombination rates, which overwhelms the distributive segregation pathway similarly to mutants that block recombination like c*(3)G* or *meiW68*. The *mtd* gene had previously been shown to be part of the Immune Deficiency (IMD) pathway, which primarily defends against gram-negative bacteria. In that study, they showed that a gain-of-function allele increased the survival rate of flies that were infected with the gram-negative bacteria *Vibrio cholerae*. These results suggest that the IMD pathway may be what modulates recombination rates in response to bacterial infection. We are testing this hypothesis by measuring if the recombination rate changes caused by *P. rettgeri* infection depends on *mtd* function.

440A Investigating chromosome-specific differences during meiosis *Katherine Billmyre*[1], R. Blake Billmyre[1], R. Scott Hawley[1,2] 1) Stowers Institute for Medical Research, Kansas City, MO, United States of America; 2) Department of Molecular and Integrative Physiology, University of Kansas Medical Center, Kansas City, KS, United States of America

Inheritance of a complete set of chromosomes is critical for fertility and production of viable offspring. However, all chromosomes are not the same. Cells face the challenge of building a system that can accurately segregate chromosomes with vast structural and size differences (as much as 5x in humans and 46x in *Drosophila*). However, the mechanisms underlying recombination and segregation of specific chromosomes are not understood. In *Drosophila melanogaster*, a set of partial loss-of-function synaptonemal complex (SC) mutants exhibit chromosome-specific defects in recombination and pairing during meiosis. The SC is a conserved meiotic structure that holds homologous chromosomes together and is necessary for the repair of double-strand breaks (DSBs) into crossovers. We are currently using a two-pronged approach of super-resolution imaging and genomics to examine the relationship between the SC, DSBs, and recombination on different chromosomes both in wildtype flies and those that exhibit chromosome-specific defects. Using next generation sequencing, we have found in a partial loss of SC background, that DSBs are able to be repaired as non-crossover gene conversions, even when they are unable to be repaired as crossovers. To further examine the importance of synapsis, pairing, DSB placement, and crossovers on the behavior of different chromosomes, we are combining the Lac*O*/Lac*I* system with super-resolution imaging and chromosome tracing to assess the meiotic landscape on individual chromosomes. Together these approaches will provide a one-of-a-kind map to elucidate the role of the SC in regulating meiosis.

441B The Effect of Heterozygous Inversion on Crossover Frequency near Inversion Breakpoints by High-Res Whole Genome Sequencing Nicole Crown, *Haosheng Li* Case Western Reserve University

Heterozygous inversions disrupt the meiotic crossover (CO) distribution by suppressing COs locally both within and outside of the inversion, and also triggering the interchromosomal (IC) effect, where the CO frequency on structurally normal chromosomes dramatically increases. We previously showed that these changes in the CO distribution are mediated by CO patterning mechanisms that alter the CO/NCO decision in *Drosophila melanogaster*. However, the exact decision making process of how the patterning mechanism exert its influence on a double-stranded break (DSB) to suppress COs outside of inversion breakpoints, as well as the pattern of suppression in terms of distance and severity, are still unknown. Traditionally, this analysis can be done by phenotypic scoring of recombinants, but we are currently analyzing the recombination mechanics of DSB repair by creating a fine-scale recombination map of the region near the breakpoints of an X chromosomal inversion dl-49 through whole genome sequencing of individual *Drosophila melanogaster* offspring. Our preliminary data show that from the distal breakpoint to telomere region, almost no COs were recovered. From the proximal breakpoint to centromere region, a bell-shaped pattern was observed, where the further away the location is from the breakpoint, the higher the CO frequency is (compared to the CO profile of completely wild type flies). To reduce variations in genomic background among tested flies, we are currently creating isogenized full-sibling stocks, and the experiment will be repeated using these new stocks for more robust data.

442C Meiotic Crossovers on Chromosome 4 induced by the Interchromosomal Effect in *Drosophila Melanogaster* *Joseph Terry*, Savana Hadjipanteli, Nicole Crown Case Western Reserve University

Meiosis is essential for the production of genetically diverse gametes in all sexually reproducing eukaryotes. During

meiosis, genetic material is exchanged between chromosomes through homologous recombination, driving genetic diversity amongst gametes while simultaneously ensuring proper chromosome segregation. Recombination is initiated in response to programmed DNA double-stranded breaks (DSBs) across the genome. These DSBs are then repaired, resulting in a crossover (CO) or non-crossover (NCO).
When looking at genome-wide DNA DSB repair distribution, NCOs make up the majority of repair outcomes while a limited number of DSBs are repaired as COs. This imbalance between the total number of DSBs across the genome and the small number of those repaired as COs introduces the question: what dictates crossover patterning across the genome? Accumulating evidence has implicated three key phenomena to be involved in crossover patterning within *Drosophila*: crossover assurance, crossover interference, and crossover suppression. Interestingly, nearly all chromosomes are affected by these three CO patterning mechanisms, with the exception of chromosome 4 where meiotic COs never occur.
Disturbances in CO patterning, such as the attenuation of CO interference and suppression have been suggested to occur during the interchromosomal effect (IC). During the IC effect, the presence of a heterozygous inversion inhibits COs locally, while increasing CO frequency across unbalanced chromosomes. Here, we propose that CO patterning mechanisms indeed, are attenuated during the IC effect by generating a fly model that undergoes meiotic COs on chromosome 4 in the presence of a multiply inverted balancer chromosome. Using a fly model that undergoes meiotic COs on chromosome 4, we aim to generate CO distribution maps as a method to assess the impact of CO patterning mechanisms on recombination during the IC effect.

443A Identification of Meiotic Recombination Nodule Proteins Utilizing Proximity Labeling *Oscar Bautista*, Haosheng Li, Gabrielle Bais, Nicole Crown Case Western Reserve University, Cleveland, OH

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 enzymatic-directed double-stranded breaks can result in crossovers (COs), involving reciprocal exchanges between homologues, or noncrossovers (NCOs), where a portion of DNA is copied from the donor homologue without altering it. Protein complexes known as recombination nodules are thought to reside around future crossovers and promote and catalyze recombination events. Although their function has been hypothesized, its protein composition has not been elucidated, nor has the mechanism through which it facilitates recombination outcomes. Additionally, due to their insoluble nature, previous attempts at isolating recombination nodules using classical biochemical purification techniques have failed. To address these issues, I will generate several fly lines containing proximity biotin-labeling protein, APEX2, conjugates of known recombination proteins. Using these fly lines and mass spectrometry, we will be able to identify novel protein interactors and create interaction maps to elucidate the proteome that is necessary for processing recombination. Utilizing our model system, I eliminate the need to isolate recombination nodules and, instead, can reliably identify protein components and interactions to understand the mechanisms that facilitate meiotic recombination. The protein-protein interactions uncovered will also provide insight into the complex regulatory mechanisms that are required to initiate double-stranded breaks and subsequently repair them as NCOs or COs.

444B Mechanism of *bruno*-mediated tolerance to *P*-element activity in *Drosophila melanogaster* germline *Modupeola Bolaji*, Erin Kelleher, Vanessa Marcano University of Houston

Transposable elements (TEs) are mobile genetic elements whose proliferation in the germline induce germ cell loss and sterility. Hosts reduce this fitness cost through resistance, which involves suppression of TE proliferation, or tolerance, where the germline withstands the damaging effects of transposition. While host resistance to TEs by piRNAs is studied extensively, little is known about host factors that confer tolerance. Our lab identified *bruno* as a source of natural variation in tolerance to *P*-element DNA transposon induced germ cell loss in *Drosophila melanogaster*. Bruno is an RNA binding protein and a translation repressor that plays important roles in the regulation of oocyte development in adults. However, the role of *bruno* in determining tolerance to transposition is unknown.
Recent studies have shown that *P*-element induced dysgenesis triggers loss of germ cells in the larval stage.
While *bruno* previously had no known function in larvae, we have discovered that Bruno protein is expressed in larval primordial germ cells (PGCs) and that Bruno function impacts *P*-element induced germ cell loss specifically in the larval stage. We are currently testing two hypotheses to explain the relationship between Bruno function and PGC loss:
1) hybrid dysgenesis increases *bruno* expression in PGCs, resulting in activation of *bruno*-dependent differentiation pathways and 2) *bruno* increases transposition of *P*-element, leading to increased DNA damage and loss of PGCs.

445C 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[2] 1) University of Illinois at Chicago; 2) 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 pole 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 during 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. These results will be compared to the known homotypic clustering and dynamics of the fly polar granules.

446A *bourbon* interacts with known germline sex determination regulator *otu* and promotes the expression of *sxl* in the *Drosophila* female germline *Marianne Mercer*, Michael Buszczak University of Texas Southwestern Medical Center, Dallas, TX

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 controls Sxl expression is less clear. Through IP-mass spec analysis we find that Otu physically interacts with Bourbon (Bbn), a previously uncharacterized protein. *bbn* displays enriched expression in germ cells, and *bbn* null mutant females exhibit 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. We tagged *bbn* endogenously with an HA tag and found it is expressed in germline stem cells, cystoblasts and two cell cysts, similar to the expression pattern of cytoplasmic Sxl protein. In addition, Bbn is enriched in developing oocytes. 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. Future experiments will help us determine if Bbn regulates Otu expression levels, localization, substrate specificity or catalytic activity.

447B Targeted mutagenesis of *orco* disrupts fertility in the second gonotrophic cycle in the *Aedes aegypti* mosquito *Olayinka David*, Kevin Sanchez, Anthony Bellantuono, Andre Costa-da-Silva, Matthew DeGennaro Florida International University

Mosquitoes are the deadliest animals to humans. They are responsible for over half a million deaths annually and get more than 200 million people around the world sick every year. Female mosquitoes of many species require vertebrate blood for egg development. A female mosquito undergoes multiple discrete rounds of reproductive cycles also known as gonotrophic cycles. A gonotrophic cycle spans the period from bloodmeal intake to the time the mature oocytes are deposited. Mosquitoes are capable of transmitting diseases to humans and other vertebrate hosts during bloodmeal intake. Transmission of mosquito-borne diseases to hosts typically occurs during the second round of blood ingestion, an event that often coincides with the start of the second gonotrophic cycle. Mosquitoes rely on odorant receptors (ORs) for detecting chemical molecules in their surroundings. This chemosensory potential is necessary for processes such as vertebrate host localization, nectar seeking, identifying suitable mates, and finding egg-laying sites. Odorant receptor co-receptor (*orco*) is an insect-specific obligate counterpart necessary for the function of tuning ORs. Studies have reported the expression of *orco* in ovaries and testes across different insect taxa. These receptors have been shown to activate both human and mosquito sperm. To determine the role of *orco* in *Aedes aegypti* reproduction, we employed individual mosquito fecundity and fertility assays to quantify the reproductive output of *Aedes aegypti orco*$^{-/-}$ mosquitoes across two gonotrophic cycles. Surprisingly, we found that eggs produced by mutant mosquitoes have a significantly reduced hatch rate in the second reproductive cycle compared to controls. Histochemical assays revealed that the unhatched eggs were fertilized but the resulting embryos failed to attain full development. Additionally, using mixed genotype mating experiments, we found that the embryo developmental defect is a maternal effect, suggesting that *orco* is required in the female mosquito for optimum embryo viability. In control experiments, we quantified the amount of ingested blood, the number of sperm stored as well as the number of eggs laid per female mosquito and found no difference between wild-type and mutant mosquitoes. Lastly, when the *orco* gene was reconstituted into the *orco*$^{-/-}$ background, the reproductive phenotype was reverted. Our findings inform a novel vector control approach that targets mosquito reproduction through halted embryo development.

448C 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[1] 1) Cornell University, Ithaca, NY; 2) College of the Holy Cross, Worcester, MA

Males transfer hundreds of seminal fluid proteins (Sfps) along with sperm to the female reproductive tract during

copulation. Sfps cause female post-mating responses, both behavioral and physiological, which ensure 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 the female flies knocked down for candidate receptors revealed several promising candidates for OvR along with others with ovulin-independent roles in ovulation. To verify direct interactions between ovulin and OvR candidates we are performing several complementary assays: membrane-anchored split-ubiquitin yeast two hybrid assays, cell-culture based assays, and in vivo approaches such as TANGO.

449A RNA–protein interaction mapping via MS2-based APEX2 targeting in the *Drosophila* ovary *Kwan Yin Lee*, Elizabeth R. Gavis Department of Molecular Biology, Princeton University, Princeton, NJ

Mechanisms of mRNA regulation commonly involve the engagement of protein factors capable of influencing transcript biology. Proteins can engage with mRNAs in stable ribonucleoprotein complexes or form more fluid and dynamic structures by coalescing into phase-separated condensates. Currently, how the physical features of these complex structures are associated with mRNA regulation remains poorly understood. We are combining proximity-dependent labeling by APEX2 with the MS2/MCP system to spatially define the molecular environment proximal to specific mRNAs in the *Drosophila* oocyte. In late-stage oocytes, *nanos* mRNA is incorporated into phase transitioned condensates known as germ granules that are a conserved feature of germline development. The *nanos* mRNAs occupying germ granules form spatially distinct clusters within the granule compartment, each containing multiple copies of the mRNA. We aim to understand the role of these clusters in mRNA regulation by defining their surrounding molecular environment using the MS2-based APEX2 targeting system described here. To test the method, we have tethered MCP-APEX2 to *nanos* mRNA. APEX2 dependent biotinylation is detected together with *nanos* mRNA at multiple sites across *Drosophila* egg chambers. Biotinylated proteins nearby *nanos* mRNA will be isolated and identified by mass spectrometry.

450B Genetic interactions between new bag-of-marbles mutants and the endosymbiont bacteria Wolbachia in D. melanogaster *Miwa Wenzel*, Charles Aquadro 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. A bam null mutant and a bam partial loss-of-function hypomorphic mutant result in sterility and reduced fertility, respectively. Like another germline gene, Sxl, bam genetically interacts with the endosymbiont Wolbachia, as Wolbachia rescues the reduced fertility of the hypomorphic mutant. Here, we explored the specificity of the bam Wolbachia interaction by combining an alanine scanning approach with phylogenetic sequence data to efficiently generate new mutants to test for Wolbachia rescue. We have generated several new mutants with reduced fertility in one of bam's documented binding regions, highlighting the functional importance of certain residues for bam's activity. All of these new reduced fertility mutants are rescued by Wolbachia in females. Additionally, we find that Wolbachia also rescues the reduced fertility of a bam transgenic allele in male D. melanogaster, revealing the first genetic interaction between Wolbachia and germline genes in males. Additional mutants in other documented binding regions of bam as well as bam RNAi knockdowns are currently being evaluated to further elucidate the specificity of the bam Wolbachia interaction. If Wolbachia rescue remains specific to this single binding region, it would suggest that the Wolbachia interaction occurs in this pathway of bam's function. Better understanding the nature of bam's interaction with Wolbachia will allow us to evaluate hypotheses about the potential contribution of Wolbachia as a driver of bam's rapid evolution in the D. melanogaster group.

451C Nuclear actin is a critical regulator of Drosophila germline stem cell maintenance *Nicole Green*, Tina Tootle Carver College of Medicine, University of Iowa, Iowa City, IA

While actin was observed in the nucleus decades ago, nuclear functions of actin dynamics have only recently become widely acknowledged. Indeed, 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 is involved in determining differentiation state, deciding cell identity, and reprogramming cells to a pluripotent state. Our studies investigate 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) reside in a niche at the anterior tip of each ovariole in a structure known as the germarium and give rise to all germline cells. GSCs are maintained by asymmetric divisions that retain a stem cell and produce a daughter cell, which continues to incompletely divide to produce 2-, 4-, 8-, and 16-cell germline cysts. Our lab previously defined several distinct pools of nuclear actin in the ovary by screening established actin labeling tools. One of these actin pools stained by anti-actin C4 is found in both the nucleoplasm and nucleolus of GSCs. These C4 positive nuclear actin pools are present in a dynamic

pattern across the germarium and are lost as cells become more differentiated. This trend suggests nuclear actin may play a role in regulating stemness. To test this hypothesis, we overexpressed NLS-actin constructs in germline cells of the germarium. When we increased monomeric nuclear actin (NLS-Act5C^{G13R}), GSC maintenance is disrupted and there is progressive germline loss which results in empty germaria. As nuclear actin is enriched in the nucleolus and previous studies uncovered that nucleolar functions are essential for GSC maintenance, we were curious whether nuclear actin regulates nucleolar functions. Using nucleolar structure as a readout for nucleolar function, we find that increasing clear monomeric actin results in increased nuclear size and hypertrophic nucleoli. These nucleoli are also deformed and fragmented suggesting altered nucleolar functions. These results indicate that nuclear actin dynamics regulate key nuclear functions, including nucleolar activity, which are necessary for maintaining stem cells.

452A Evaluating the Effect of Architectural Features on Border Cell Migration in Drosophila *Alexander George*, Bradford Peercy, Michelle Starz-Gaiano University of Maryland Baltimore County

Collective cell migration is paramount throughout a multicellular organism's life from embryogenesis to adult tissue maintenance. Many cell migration studies are conducted *in vitro*, but to understand the full complexity of the living tissue environment, more *in vivo* studies are required. Using the border cells, which navigate as a cluster through the 3-D terrain of the *Drosophila* egg chamber during oogenesis, we can investigate the impact of physical architecture on collective cell migration and migration-related signaling *in vivo*. We observe small, acellular gaps at cell-cell junctures along the border cell migration route and hypothesize that these architectural features directly affect the migration behaviors of the border cells, in part by altering local distributions of secreted chemical attractants (chemoattractants). *In silico*, we have demonstrated that these gaps can affect the distribution of the morphogen that specifies border cell fate, as well as the distribution of secreted chemoattractants. Using staining and imaging techniques, we characterized the geometry and distribution of these gaps in fixed tissue and observed changes in border cell behavior at cell-cell junctures where gaps are observed in live tissue. Furthermore, flooding these gaps with above-endogenous levels of chemoattractant delayed border cell migration, and thus we are considering the effect of receptor dynamics on cluster migration. Additionally, we are using two complementary methods to reveal the chemoattractant gradient and evaluate the influence of the egg chamber's terrain on signal distribution and consequently, border cell migration. First, by inducing extra border cells throughout the egg chamber and examining their speed and persistence, we infer the positions of local hotspots of concentrated chemoattractant. And second, by tagging chemoattractants to visualize them, we can examine signal distribution in real-time. This data will further inform and allow us to refine our *in silico* models on how border cells might respond to the chemoattractant gradient. Our studies emphasize the importance of considering physical features of the tissue architecture when examining collective migration and migration-relevant signaling *in vivo.*

453B A Genetic Screen Identifying E2s and E3s Involved with Maternal Protein Clearing During the Maternal to Zygotic Transition *Calvin Bleskan*[1,2,3], Chloe Briney[2,3], Jesslyn Henriksen[2,3], Olivia Rissland[2,3], Hector Cobian[1,2,3] 1) Metropolitan State University, Denver, CO, USA; 2) Department of Biochemistry and Molecular Genetics, University of Colorado School of Medicine, Aurora, CO, USA; 3) RNA BioScience Initiative, University of Colorado School of Medicine, Aurora, CO, USA

The maternal-to-zygotic transition (MZT) is an essential process during early animal embryogenesis where developmental control shifts from maternally deposited gene products to a newly made zyogtic one. Although the MZT typically considered from the perspective of RNA, we and others have found that maternal protein clearance is a key part of this transition. As part of a multi-year undergraduate research project, we are performing an RNAi screen to identify E2s and E3s required for oogenesis and embryogenesis. This RNAi screen consists of expressing RNAi constructs using an ovary-specific UAS driver and of quantifying various phenotypes from these female flies (including egg laying, egg viability, and other phenotypes). Here, we will describe the results for ten randomly selected genes: skp2, cul-1, Elo-C, Gus, SAK, CSN5, cul-2, skpA, UBC-4, and UBC-D6[BC1] . Preliminary results indicate that knock down of cul-1 resulted in lethality for a majority of embryos. Because Cul-1 is a component of the SCF E3 complex and the SCF complex regulates cell cycle progression and the removal the Smaug RNA binding protein, this result is consistent with Cul-1 performing an essential role during embryogenesis, but not oogenesis. Interestingly, embryos with UBC-4 depletion showed a developmental delay in pupae eclosation, although the underlying mechanisms are unknown. Together, these results demonstrate the potential of this screen to provide a foundation for future research on protein clearance during the MZT.

454C A Genetic Screen for Identifying E2s and E3s Involved in Protein Clearance During the Maternal-to-Zygotic Transition *Hector Cobian*[1,2,3], Chloe Briney[1,2], Jesslyn Henriksen[1,2], Calvin Bleskan[1,2,3], Olivia Rissland [1,2] 1) RNA BioScience Initiative, University of Colorado School of Medicine, Aurora, CO ; 2) Department of Biochemistry and Molecular Genetics, University of Colorado School of Medicine, Aurora, CO; 3) Metropolitan State University of Denver, Denver, CO

The maternal-to-zygotic transition (MZT) is an essential developmental process whereby maternal gene products deposited into the oocyte are degraded, and zygotic gene products take their place. The degradation of maternal RNA has been well studied and is known to be an important part of the MZT. However, the removal of maternally deposited proteins is far less studied. The main mechanism for protein degradation pathway works through the

ubiquitin-proteosome system, which uses E1s, E2s, and E3s and culminates in the ubiquitylation of target proteins, thus marking them for degradation. The *Drosophila melanogaster* gene encodes dozens of E2s and hundreds of E3s, but we know very little about their involvement in the MZT. As part of a multi-year undergraduate research program, we are performing an RNAi screen to identify E2s, E3s, and related proteins that are essential for oogenesis and embryogenesis. Here, we will describe the results of ten randomly selected E2 and E3 genes: *CG11658*, *Cul-3*, *Cul-5*, *EloB*, *faf*, *slmb*, *l(3)73Ah*, *CSN6*, *Nedd4*, and *Prosalpha5*. We will present results on the effectiveness of RNAi knockdown by RT-qPCR and western blotting. We will describe the extent to which each gene affects: egg laying; embryonic viability; other developmental effects. In the future, we will be able to use unbiased techniques, like mass spectrometry, to identify putative targets of maternally required E2s and E3s. Together, this screen will help reveal the factors required for proper protein degradation in the MZT.

455A Octopaminergic/tyraminergic *Tdc2* neurons regulate sperm preference in female *Drosophila melanogaster* *Dawn Chen*, Andrew Clark, Mariana Wolfner Cornell University, Ithaca, NY

In polyandrous internally fertilizing species, a multiply-mated female can exert preference on which stored sperm she uses to fertilize her eggs. The female's ability to assess sperm quality and compatibility is essential for her reproductive success, and represents an important aspect of postcopulatory sexual selection. In *Drosophila melanogaster*, previous studies demonstrated that the female nervous system plays an active role in influencing progeny paternity proportion, and suggested a role for octopaminergic/tyraminergic *Tdc2* neurons in this process. Here, we report that inhibiting *Tdc2* neuronal activity causes females to produce a higher-than-normal proportion of first-male progeny. This difference is not due to differences in sperm storage or release, but attributable to the suppression of preference for second-male sperm that normally occurs in control females. We further show that a subset of *Tdc2* neurons innervating the female reproductive tract was largely responsible for the progeny proportion phenotype that was observed when *Tdc2* neurons were inhibited globally. On the contrary, over-activation of *Tdc2* neurons does not further affect sperm storage and release or progeny proportion. These results suggest that octopaminergic/tyraminergic signaling allows a multiply-mated female to exert sperm preference, and identify a new role for the female nervous system in postcopulatory sexual selection.

456V Transcriptional and mutational signatures of the aging germline Evan Witt, Christopher Langer, *Li Zhao* Laboratory of Evolutionary Genetics and Genomics, The Rockefeller University, New York, NY

Aging is a complex biological process which is accompanied by changes in gene expression and mutational load.In many species including humans, old fathers pass on more paternally-derived *de novo* mutations, however, the cellular basis and cell types driving this pattern are still unclear. To understand 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 tissue from the same flies. We found that early germ cells from old and young flies have similar mutational loads, but older flies are less able to remove mutations during spermatogenesis. This indicates that germline mutations arise from primarily non-replicative factors, and that the increased mutational load of older males is due to differences in genome maintenance activities such as repairs to DNA damage. We also found that T>A mutations are enriched in older flies, and transcription-related enrichment terms are depleted in older males. Early spermatogenesis-enriched genes have lower dN/dS than late spermatogenesis-enriched genes, supporting the hypothesis that late spermatogenesis is the source of evolutionary innovation. This transcriptional disruption is reflected in the decreased expression of genome maintenance genes in early germ cells of older flies, as well as potentially aberrant transcription of transposable elements in the aging germline. Our results provide novel insights into the transcriptional and mutational signatures of the male germline.

457V Modeling effects of human disease variant of Barrier-to-Autointegration on oogenesis *Felipe Rodriguez*[1], Tingting Duan[1], Katherine Mathews[2], Pamela Geyer[1] 1) University of Iowa. Department of Biochemistry and Molecular Biology; 2) University of Iowa. Stead Family Department of Pediatrics

The nuclear envelope is a complex structure that defines the cellular nucleus. Lying beneath the inner nuclear envelope is the nuclear lamina (NL), a protein network comprised of lamins and lamin-associated proteins. One abundant protein in this network is the Barrier-to-Autointegration Factor (BAF), a small dimeric protein that binds the NL LEM domain proteins, histones, and DNA, all properties that contribute to the organization of chromatin within the nucleus. Mutations in the amino terminus of BAF cause human diseases associated with age-related declines in tissue function, including Nestor Guillermo progeria (NGP) and neuromuscular disease (NMD). To gain insights into the mechanism of these diseases, we are modeling disease associated mutations in Drosophila BAF, a protein with 63% identity to human BAF. We are investigating the effects of the disease variants of BAF on the ovary, as BAF is essential for sustained oogenesis. We find that oogenesis is impaired in NGPs flies, with females laying few eggs that do hatch. Our data suggest that reduced egg production is linked to altered GSC mitosis, leading to increased DNA damage and death of transit amplifying cells, defects associated with activation of the DNA damage response kinase Checkpoint kinase 2. We are initiating studies of the NMD variant. Our Drosophila studies of the NMD variant will build from our observations that

NMD patient fibroblasts display age-dependent increases in nuclear ruffling, higher levels of heterochromatin, and increased DNA damage, defects linked to changes in the cell cycle that lead to faster proliferation. Taken together, our studies will provide an understanding of the role of BAF in development and tissue homeostasis.

458V Polycomb group (PcG) proteins prevent the assembly of higher order repetitive structures during meiosis Bruno Marques[1], Tália Feijão[1,3], Rui Silva[1,2], Daniel Sobral[4], Ricardo Matos[1], Célia Carvalho[5], Antonio Pereira[3], Eurico Morais de Sá[3], Hélder Maiato[3], *Rui Gonçalo Martinho*[1,5,6] 1) Algarve Biomedical Center Research Institute (ABC-RI), Universidade do Algarve, 8005-139 Faro, Portugal; 2) Faculty of Medicine and Biomedical Sciences (FMCB), Universidade do Algarve, 8005-139 Faro, Portugal; 3) i3S - Instituto de Investigação e Inovação em Saúde, Universidade do Porto, 4200-135 Porto, Portugal; 4) Associate Laboratory i4HB - Institute for Health and Bioeconomy, and UCIBIO - Applied Molecular Biosciences Unit, Department of Life Sciences, School of Science and Technology, NOVA University Lisbon, 2819-516 Caparica, Portugal; 5) Instituto de Medicina Molecular João Lobo Antunes, Faculdade de Medicina, Universidade de Lisboa, 1649-028 Lisboa, Portugal; 6) Department of Medical Sciences and Institute for Biomedicine (iBiMED), Universidade de Aveiro, 3810-193 Aveiro, Portugal

The synaptonemal complex (SC) is a proteinaceous scaffold that is assembled between the paired homologous chromosomes during the onset of meiosis. The SC stabilizes chromosome pairing and is important for crossovers formation, recombination and accurate segregation of meiotic chromosomes. Timely expression of SC genes is essential for SC assembly and successful meiosis. However, SC components have an intrinsic tendency to self-organize into alternative higher order repetitive structures, which are not assembled between the paired homologs and whose formation is potentially deleterious for meiosis and gametogenesis. This creates an interesting conundrum, where SC genes need to be robustly expressed during mitotic to meiotic transition, but their expression must be carefully regulated to prevent the formation of abnormal SC structures. In this manuscript, we show that the Polycomb group protein Sfmbt, the *Drosophila* ortholog of human MBTD1 and L3MBTL2, is required to avoid excessive expression of SC genes during prophase I. Although SC assembly is normal after Sfmbt depletion, SC disassembly is abnormal with the formation of a complex network of alternative SC structures (polycomplexes) within the oocyte. Overexpression of the SC gene *corona* and depletion of other polycomb group proteins are similarly associated to polycomplexes formation during SC disassembly. These polycomplexes are highly dynamic and have a well-defined periodic structure. Further confirming the importance of Sfmbt for female gametogenesis, germ line depletion of this protein is associated to significant metaphase I defects and a reduction of female fertility. Polycomb group proteins are therefore crucial for coherent gene expression during prophase I.

459V Identification of factors regulating individualization. *Sepideh Dadkhah*, Douglas Harrison University of Kentucky, Lexington, KY

Sperm development in *Drosophila* has many similarities to spermiogenesis in other organisms. Individualization is one of the later stages of spermiogenesis in which the 64 interconnected spermatids in a cyst are separated and excess cytoplasm eliminated. The synchronous differentiation of spermatids from interconnected spermatocytes is evolutionarily conserved. While JAK/STAT pathway activation in the somatic cyst cells is required for individualization, the mechanism by which it controls the process is not known. The aim of this project is to identify effectors regulated by JAK/STAT signaling that initiate individualization.
Using RNA-seq, we have examined expression profiles from testes in which JAK/STAT signaling has been impaired prior to individualization by expressing the negative regulator Eye-transformer/Latran (ET/Lat) and compared to testes dissected from wild type flies. Estimates of transcript abundance of the control and experiment profiles were calculated. Differential expression analysis identified more than 400 genes that were differentially expressed upon the arrest of JAK/STAT signaling at elongation. The differentially expressed genes identified in the RNA-Seq analysis were prioritized based on being associated with relevant GO terms and relation to the JAK/STAT pathway. Knockdown of 38 prioritized genes was carried out by utilizing the UAS-Gal4 system to express corresponding RNAi constructs with somatic and germline drivers. Functional analysis was done by quantitative analysis of elongated spermatid nuclei bundles and investment cones, both hallmark events of progression of individualization. From this functional analysis, genes involved in extracellular matrix and cell signaling were found to influence individualization.

460V Distinct downstream effectors downstream of InR activity control multiple aspects of oogenesis. *Tancia Bradshaw*, Alissa Armstrong University of South Carolina

Approximately 40% of adults in the United States are obese. Adipocytes, the primary cellular component of fat tissue, are nutrient-sensitive and secrete adipokines that control physiology, including metabolism, appetite, and insulin sensitivity. Despite the intricate relationship between dietary input, adipose tissue, and peripheral organ function, we are at the tip of the iceberg regarding our understanding of the cellular and molecular mechanisms underlying adipose communication to other tissues. The *Drosophila* ovary receives nutritional signals from the fat body with multiple nutrient-sensing pathways functioning in adipocytes to control oogenesis. Insulin/insulin-like growth factor signaling (IIS) within adult adipocytes remotely controls oocyte production at distinct stages of oogenesis. The PI3K/Akt1 axis in

adipocytes promotes germline stem cell (GSC) maintenance via SGG, *Drosophila* GSK3beta, yet its targets in this context remain to be identified. Here, we measured the expression of candidate SGG/GSK3beta substrates in adipocytes and assessed their role in regulating GSC number. Using the *UAS/Gal4* system for adipocyte-specific knockdown of four putative targets, *ATPCL*, *chb*, *porin*, and *SREBP*, we find that *SREBP* acts within adipocytes to regulate GSC maintenance. In addition, we find that a second axis downstream of the insulin receptor, the Ras/MAPK signaling pathway, acts cell autonomously to control adipocyte size and remotely to regulate the survival of early and late germline stem cell progeny – germline cysts and vitellogenic egg chambers, respectively. Future studies will identify how SREBP activity within adipocytes controls GSC maintenance as well as additional SGG/GSK3beta substrates within adipocytes required to communicate to the ovary. These studies highlight the complex mechanisms that underlie inter-organ communication.

461V Warm and cold temperatures have distinct germline stem cell lineage effects during *Drosophila* oogenesis *Ana Caroline Gandara*, Daniela Drummond-Barbosa Johns Hopkins University, School of Public Health, Baltimore, MD

Reproduction of all organisms is shaped by their external environments. As climate change alters the abundance and distribution of many types of organisms around the globe, investigating how temperature impacts the reproduction of organisms has become a major area for scientific investigation. Insects are cold-blooded animals and therefore particularly sensitive to environmental temperatures. Oogenesis is highly sensitive to a variety of environmental and physiological factors; however, it remains largely unknown how exposure of *Drosophila melanogaster* adult females to chronic thermal stress affects oogenesis. To directly address this question, we incubated newly-eclosed *y w* adult flies (raised at room temperature, 23°C) at 18°C (cold), 25°C (optimal), or 29°C (warm) for 20 days at constant humidity (>70%) and found that the rate of egg production was reduced in females at both warm and cold temperatures, albeit through distinct cellular mechanisms. Chronic exposure of females to 18°C improved the maintenance of germline stem cells, survival of early germline cysts, and hatching rates but reduced the rate of follicle growth with no obvious effect on vitellogenesis. By contrast, in females at 29°C, germline stem cell numbers and follicle growth were comparable to those in females at 25°C, while early germline cyst death and degeneration of follicles were markedly increased, and hatching rates plummeted over time. Finally, we uncovered a novel role for the warm temperature receptor TrpA1 in mediating the effects of 29°C on oocyte quality. These findings are widely relevant not only to cold-blooded organisms, which exhibit limited thermoregulation, but also potentially to warm-blooded organisms, which are subject to hypothermia, heatstroke, and fever.

462V Obesity and oogenesis in *Drosophila*: Increased fat storage is not sufficient to impair fertility *Rodrigo Dutra Nunes*, Daniela Drummond-Barbosa Johns Hopkins Bloomberg School of Public Health

Obesity is highly correlated with infertility; however, the underlying mechanisms remain largely unknown. Our previous research revealed complex roles of adipocytes in multiple steps of *Drosophila* oogenesis, and others have shown that a high sugar diet (HSD) causes obesity and reduces egg production. To carefully investigate how obesity affects oogenesis, we increased dietary sugar content from 5% (ND) to 30% (HSD). In accordance with published studies, females on HSD had a 3-fold increase in triacylglycerol content and lipid droplets, a 2.5-fold increase of glycogen content, and a drastic reduction in egg production and quality compared to ND controls. In addition, we found that these obese females ingested 5 times less food and had increased death of early germline cysts and vitellogenic follicles. In these experiments, however, HSD drives obesity, and it is not clear whether changes in oogenesis are due to HSD, obesity, or both. To test how obesity alone impacts oogenesis, we induced adult adipocyte-specific RNAi against the *brummer* or *adipose* genes (both of which inhibit excess fat accumulation). Adipocyte-specific *brummer* knockdown led to a marked increase in triacylglycerol content and adipocyte lipid droplet size, comparable to those observed in HSD-induced obese females. Strikingly, these highly obese females did not show any significant differences in food ingestion, glycogen content, specific steps of oogenesis, rates of egg production, or oocyte quality relative to non-obese control females. We obtained similar results in obese females generated by adipocyte-specific RNAi against *adipose*. These results indicate that increased fat storage is not sufficient to reduce fertility. It remains unclear, however, if obesity is required for the reduced fertility of HSD-induced obese females: it is possible that either HSD alone or in combination with obesity is responsible for their fertility defects. Our future studies will investigate the underlying mechanistic basis for the oogenesis differences we observe in these different types of obese females.

463V Validation of candidate genes influencing egg size in cold-adapted *Drosophila melanogaster* *Cecelia Miles*[1], Allison Hoefakker[1], Intisar Koch[1], Rachel Flynn[2], Rosanna Beraldi[1] 1) Augustana University, Sioux Falls, SD; 2) Mount Marty University, Yankton, SD

Egg size is a highly polygenic trait that is closely related to fitness in *Drosophila melanogaster.* Cold-adapted *D. melanogaster* are known to produce larger eggs (Azevedo et al. 1996). The Miles lab previously identified 22 candidate genes influencing egg size based on whole-genome sequence data in multiple cold-adapted lines. Here we used semi-quantitative PCR to try and validate five of our 22 candidates (*cappuccino, stathmin, dreadlocks, lilliputian,* and gene *CG31690*) by comparing expression in cold-adapted inbred lines to the standard lab line OregonR. Expression differences in *stathmin* were found in a majority of the cold-adapted lines and appear to represent the absence of a

splice variant. This is the first validation of a gene from our candidate list. We also report a significant upregulation in actin observed in half of our cold lines. We are currently following up on these results using qPCR.

464V Genetic Requirement of IC effect *Bowen Man*, Nicole Crown Case Western Reserve University

While the interchromosomal effect (IC effect) has been well characterized at the phenotypic level, almost nothing is known about the mechanism. Crossovers that occur during meiosis require a specific set of proteins to form and are subject to crossover control mechanisms such as interference (these are the so-called Class I COs). However, COs can also form using mitotic-like CO pathways, but these use a different set of proteins and are not subject to crossover control mechanisms. I will be determining if the extra COs that form during the IC effect are Class I meiotic COs or the mitotic-like COs. To do this, I'll measure the IC effect in *mei-9* mutants and *blm* mutants. Evidence suggests that mei9 is playing a critical role in resolving recombination intermediates into COs during meiotic recombination by cleaving double Holiday junctions into COs. Therefore, the mei9 mutant would not be able to produce class I crossover. By comparing the CO distribution of mei9 mutant and mei9; +/TM6B, the identity of extra crossovers from IC effect can be determined. Blm mutant would produce crossovers that lack chromosomal interference, so those crossovers found in blm mutants are considered to be class II crossovers. Therefore, if the blm mutants with balancer are scored, I can further insight about those extra crossovers from IC effect.

465V Broad is sex and cell type specifically required in the Drosophila gonads for gametogenesis and fertility. *PRADEEP BHASKAR*, Brian Oliver NIDDK, NIH

Broad (Br, CG11491, FBgn0283451) is a BTB domain (Broad-Complex, Tramtrack and Bric a brac) containing protein that has a homodimerization domain at the N terminus and multiple copies of either zinc fingers of the C2H2 type or Kelch repeats. Br is best known as a modulator of the ecdysone-response and has been attributed to cause chromatin changes by puffing of chromosomes to affect gene expression. We examined *Br* expression and function in third instar larval gonads when ecdysone levels are high.
In our whole gonad RNA-seq experiments, we detected 11 out of 15 Br transcripts encoding 4 different protein isoforms of Br (Z1-Z4), on the basis of distinct Zinc-fingers at C-terminus. Further, Single Cell RNA sequencing (scSeq) and immunostaining with antibody show that, Br, was expressed only in somatic cells of testis and ovary. In the testis, Br expression was enriched in cyst cells that enclose the spermatogonia, which are mitotic germline cells near the apex, but not the cyst cells surrounding spermatocytes. Br was also expressed in terminal epithelium, which ultimately attaches to the reproductive tract derived from the genital disc, and the pigment cells that ensheath the testis. In ovary, we found Br expression in all somatic cells (Sheath cells, Terminal Filament, Cap cells, Intermingled cells, follicle cell and Swarm cells). To determine the function of Br in these cell types we knocked down of all of its isoforms (by targeting common BTB domain) in the enclosing the germ cells (male cyst cells and female intermingled cells) using a *traffic jam* driver. This led to female-specific sterility due to germline defects including loss of germ cells. Br expression was lost from the spermatogonia cyst cells, but did not result in an overt phenotype. However, knockdown of Br using a *doublesex* driver resulted in sterility in both sexes. Knockdown of individual isoforms (RNAi against C-terminus) using *doublesex* driver results in testes that failed to elongate and attach to the reproductive tract, while in females egg retention phenotype was observed. Both males and females from these crosses were sterile. This indicates that Br is required in terminal epithelium to mediate attachment to the reproductive tract as well as egg laying in females and leads to sterility when Br is knocked down. Interestingly we found an enhancer that drives Br expression in both sexes, indicating dual role of enhancer in regulating Br function in sex and cell type specific way. In summary, we show that Br is required in both sexes, but in fundamentally different somatic cell types. This highlights the context-dependency of gene expression in sexual development and fertility.

466V Nucleoporin107 mediates female sexual differentiation via Dsx *Offer Gerlitz*[1], Tikva Shore[1], Tgst Levi[1], Rachel Kalifa [1], Amatzia Dreifuss [1], Dina Rekler[1], Ariella Weinberg-Shukron[2], Yuval Nevo [3], Tzofia Bialistoky [1], Victoria Moyal [1], Merav Yaffa Gold [1], Shira Leebhoff [1], David Zangen [4], Girish Deshpande [5] 1) IMRIC, The Hebrew University- Faculty of Medicine; 2) Weizmann Institute of Science, Rehovot, Israel; 3) The Hadassah Hebrew University Medical Center, Jerusalem, Israel; 4) Division of Pediatric Endocrinology, Hadassah Hebrew University Medical Center, Jerusalem, Israel.; 5) Princeton University, Princeton, NJ, USA

We previously identified a missense mutation in the nucleoporin-107 (Nup107; D447N) as the cause for XX-ovarian-dysgenesis in five female cousins from a consanguineous family. All men in the family had normal pubertal development and those married have multiple children. Nup107 is an essential component of the nuclear pore complex expressed ubiquitously in a sex-non-specific manner. Modelling of the human mutation in Drosophila or specific knockdown of Nup107 in the gonadal soma resulted in ovarian-dysgenesis-like phenotypes, while male flies were fully fertile.
To uncover the targets of Nup107 that mediate its sex and tissue specific activity, we conducted a genome-wide transcriptomic analysis on larval gonads compromised for Nup107. We identified 82 candidate genes which displayed significant changes in mRNA expression, including doublesex (dsx), the somatic sex-determination gene. Either loss or gain of Dsx in the gonadal soma is sufficient to mimic or rescue the phenotypes induced by Nup107 loss, establishing Dsx

as a primary relevant target of Nup107. 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. We also uncovered the cellular impairments underlying BMP signaling dysregulation. 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.

467V Tudor5-like promotes post-transcriptional regulation of maternal RNAs *Caitlin Pozmanter*, Sydney Kelly, Harrison Curnutte, Mark Van Doren Johns Hopkins University

Tudor-domain containing proteins are conserved across the animal kingdom for their necessary functions in germline development including post-transcriptional gene regulation. Recent work in our lab identified Tudor5-like (Tdrd5l), which promotes male germline identity in germline stem cells (GSCs) in the testis, but is repressed by the RNA binding protein Sex lethal (SXL) in female GSCs. Interesting, Tdrd5l is also expressed in the differentiating germline in both sexes, indicating that it may also act to control germline differentiation in both sexes.
Previously we reported that Tdrd5l localizes to an RNA granule. To understand what RNA regulatory pathway Tdrd5l functions in, we conducted RNAi against the deadenylase *twin* in mutant gonads, which revealed a genetic interaction between *Tdrd5l* and the CCR4-NOT deadenylation complex. Recent investigation into the role Tdrd5l plays in the female germline showed a decreased hatch rate and dorsal appendage defects in eggs laid by *Tdrd5l* mutants. Since Grk regulates dorsal appendage development we stained for Gurken(Grk) in *Tdrd5l* mutant ovaries and wild type ovaries. In wild type flies we see normal Grk translation in the anterior dorsal corner of the oocyte, while in *tdrd5l* mutant ovaries we see translation of Grk in the nurse cells. A similar nurse cell expression phenotype is observed when immunostaining for Orb in *Tdrd5l* mutants which is a known activator of Grk translation. This suggests the loss of maternal RNA regulation by Tdrd5l could result in patterning defects. Additionally, both *osk* mRNA and protein are mislocalized in *Tdrd5l* mutant oocytes where we observed them localized to the center of the oocyte instead of the posterior. One possibility is Grk fails to specify the posterior before migrating to the dorsal anterior corner. To investigate if this was due to failure to specify the posterior pole we used kin-lacZ and found in *Tdrd5l* mutants, kinesin does not strictly localize to the posterior pole of the oocyte in stage 9 or later egg chambers. Together our results indicate that Tdrd5l functions in regulating maternal RNA repression. To determine the mechanism by which this occurs we are currently conducting proximal biotinylation to identify other proteins in the Tdrd5l granule.

468V The bHLH-PAS transcriptional complex Sim::Tgo plays active roles in late oogenesis to promote follicle maturation and ovulation *Rebecca Oramas*[1], Elizabeth Knapp [1], Jianjun Sun[1, 2] 1) Department of Physiology & Neurobiology, University of Connecticut; 2) Institute for Systems Genomics, 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 follicle cells to release encapsulated oocytes. In order for follicles to ovulate successfully, follicles need to become mature and gain ovulatory competency. Our previous work showed that upregulation of Zinc finger transcription factor Hindsight (Hnt) in follicle cells of stage-14 *Drosophila* egg chambers induces the expression of octopamine receptor in mushroom body (OAMB) in all follicle cells and matrix metalloproteinase 2 (MMP2) in posterior follicle cells, both of which are essential for ovulation. We also found that NR5A-family nuclear receptor Ftz-f1 is upregulated in stage-10 follicle cells and promotes follicle maturation via bHLH/PAS-family transcription factor Single-minded (Sim). It is largely unclear how Sim, a critical player in central nervous system development, promote follicle maturation and ovulation. In this work, we discovered that Tango (Tgo), a class-II bHLH/PAS-family transcription factor and cofactor of Sim, is also expressed in follicle cells during late oogenesis. In addition, Tgo relocates to the nucleus when Sim is co-expressed in follicle cells from stage 10-12 and stage 14. Genetic manipulation suggests that Tgo is essential for follicle cell differentiation as Sim. Furthermore, we discovered that reupregulation of Sim in stage-14 follicle cells is also essential for ovulation in addition to its role in stage 10-12 follicle cells with Tgo. This late Sim not only induces expression of Hnt, OAMB, and MMP2, but also NADPH oxidase (Nox), another factor involved in superoxide production and ovulation. Together, our work indicates that Sim and Tgo play multiple roles in late stage follicle cells to promote follicle maturation and ovulation.

469V Explore the roles of steroid hormone signaling mediated *Drosophila* oogenesis *Chueh Wen Wang*, Anna C.-C. Jang Biotechnology and Bioindustry Sciences, National Cheng Kung University, Tainan, TW

Steroid hormone is temporal control in development process. The p160 steroid receptor coactivators (SRC) are shown being amplified and overexpressed in various cancers to promote proliferation and metastasis. However the molecular mechanism by which SRC induces dissemination of tumors has not been well established. Therefore, use border cells that are originated from follicle epithelial cells and invade through nurse cells that migrate to oocyte boundary in *Drosophila* oogenesis be study collective cell migration model. This migration process is also regulated by steroid hormone signaling and required SRC, named Taiman (Tai) in *Drosophila*. Tai interact with hormone receptor complex via LXXLL domain in a ligand dependent manner. Without any of these components, border cells fail to migrate and egg

chambers will not develop. Steroid hormone in Drosophila is spatially and temporally regulated in oogenesis and this signaling is required to schedule border cell migration. Steroid hormone signaling begins to rise during stage 9 then reaches the peak at stage 10 and its level is controlled by the bHLH domain of Tai. The bHLH domain truncated Tai, Tai (ΔB), causes hyperactivation of steroid hormone signaling and precautious migration in co-expression of hyperactive Jak. To further explore the molecular mechanism by which Tai (ΔB) regulates steroid hormone signaling, we screened for genetic modifiers of *tai (ΔB)* by crossing with deficiency lines that include various deletions on chromosome II. In overexpression of *tai (ΔB)*, nearly 60 % border cell failed to arrival at the oocyte and 15 % border cells did not exceed a quarter of migration journey. I have screened 209 deficiency lines to seek any of them to enhance or suppress migration defect. Currently, there are 48 lines displaying suppression phenotype and 74 lines showing enhancement. We will further analyze the top 10 enhancers and suppressors to identify which genes are responsible for the migration defect in hyperactivation of steroid hormone signaling. To figure out which gene involved in spatial regulation of cell migration.

470V Functions and interactions of sperm-bound seminal proteins in *Drosophila melanogaster* *Sarah Allen*, Snigdha Misra, Mariana Wolfner Cornell University, Ithaca, NY

Sperm enter mating Drosophila females accompanied by secretions from the male reproductive tract. These secretions include seminal fluid proteins (SFPs) that modify the physiology of the female to enhance the fertility of the mated pair. While most seminal proteins are present transiently within the mated female, a few bind to sperm. In particular, the seminal "Sex Peptide" (SP) remains bound to sperm long-term, allowing it to be retained to modulate female behavior and physiology for ~10-14 days. To determine the generality of this response, we collaborated with the Dorus and Pitnick labs* to identify sperm-bound SFPs in an unbiased mass spectrometry screen. We are using RNAi to determine the fertility functions of sperm-bound SFPs detected in this screen. In addition, we are using two tagging methods (TAP, TurboID) to identify the binding targets of one of these SFPs (SP) on sperm.

*Whittington, Murphy, Singh, Pitnick, Wolfner, and Dorus, The life history of Drosophila sperm involves molecular continuity between male and female reproductive tracts. In review

471V Female factors are important for the seminal Sex Peptide's association with sperm, in mated *D. melanogaster* *Snigdha Misra*[1], Akanksha Singh[2], Mariana Wolfner[1] 1) Cornell University; 2) National Heart Lung and Blood Institute, NIH, Bethesda, MD

Seminal fluid proteins (SFPs) induce a myriad of physiological and behavioral changes in mated female flies that are needed for efficient fertility. These post-mating changes last for ~10-14 days, because some male-derived seminal proteins (LTR-SFPs) "prime" sperm to bind the seminal Sex Peptide (SP). This allows SP to persist in the female and modulate post mating responses long-term. All factors currently known to account for binding of SP to sperm within the female reproductive tract are male derived. We wished to know whether female factors (protein/non-protein) also play roles in associating SP with sperm. We found that sperm in ejaculate bind SP much more weakly than sperm within females, suggesting that priming of sperm improves inside the female reproductive tract. Moreover, SP binding to sperm increases with time or transit within the female reproductive tract. Interestingly, while some LTR-Sfps also bind to sperm, the effects of female factors on the timing of their (transient) sperm-binding differ. This, in turn, suggests that female molecules participate in facilitating SP's and other SFPs' binding to sperm. Ablation of the female's spermathecal secretory cells (SSCs), or mutation of *Hr39*, which leads to defective SSCs and/or parovaria, did not affect SP's initial binding to sperm, although *Hr39* mutant females retained more sperm and SP long-term.

472C Examining essential functions of KDM5 via a novel truncation allele *($kdm5^{Q19}$)* *Melissa Castiglione*[1], Hayden Hatch [1], Julie Secombe[1], Andreas Bergmann[2] 1) Albert Einstein College of Medicine, Bronx, NY; 2) UMass Chan Medical School, Worcester, MA

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 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.

473A Use of transformants bearing deletions in the 5' upstream region of the *Hdc* gene to identify regions required

for CNS expression of *Hdc* *Collin Louis*[1], Martin Burg[1, 2] 1) Dept. of Biomedical Sciences, Grand Valley State University, Allendale, MI; 2) Dept. of Cell & Molecular, Grand Valley State University, Allendale, MI

The study of histamine and its function in *Drosophila melanogaster* has primarily been focused on its role as a neurotransmitter, both in photoreceptors and neurons of the CNS, leading to the identification of histamine's role in a number of functions such as vision, grooming behavior, thermal preference, and sleep[1]. Histidine decarboxylase is the enzyme that synthesizes histamine, and is encoded by the *Hdc* gene. Mutations in the *Hdc* gene have been identified that result in the inability to synthesize histamine in the entire fly[2]. We have set out to determine how tissue-specific expression of *Hdc* could be regulated by creating deletions in the 5' region of the *Hdc* gene where 3 distinct transcription start sites (TSSs) have been reported for *Hdc*[3]. A 9.4 kb genomic Xba1 fragment was cloned into the P{CaSpeR-3} vector and used to generate germline transformants (P{CaSpeR3-$Hdc^{9.4+}$}) in a mutant Hdc^{JK910} background. Histamine immunostaining of the P{CaSpeR3-$Hdc^{9.4+}$} transformant indicated that all CNS neurons contained histamine in both larvae and adults, suggesting that the *Hdc* gene was intact. A series of deletions, in ~300 bp steps, were generated in the 9.4 kb Xba1 *Hdc* genomic fragment from the 5' end (~4.3 kb from the start of the HDC coding region) towards the coding sequence and transformed back into Hdc^{JK910} mutant flies. Various P{CaSpeR3-$Hdc^{\Delta x}$} transformants were subjected to histamine immunodetection to determine which histaminergic cells in the CNS were no longer detected as each successive deletion step was taken in the transgene. Larval and adult stages were examined for alterations in the CNS and PNS pattern of histamine staining to correlate expression in specific cells with the removal of genomic regions containing predicted TSSs. Deletions that removed the *Hdc*-RD TSS had minimal effect in the tissues examined, while the removal of the TSS associated with the *Hdc*-RB, -RC isoforms appeared to cause loss of histamine staining in most of the CNS cells, while leaving PNS expression (photoreceptors) intact. Additionally, we have identified 3 consecutive deletions that disrupt expression in single pairs of neurons that span between the Hdc-RD and Hdc-RB, -RC isoform TSSs[3]. Elimination of these specific regions in the 5' region of the *Hdc* gene do have incremental effects on *Hdc* expression, suggesting that some of the TSSs identified are tissue-specific. These transgenic deletion-bearing flies could be useful as tools for examining the contribution of some histaminergic CNS neurons to a variety of behaviors ascribed to histamine in the CNS as photoreceptor expression appears to be retained as the *Hdc*-RA TSS was not disrupted.

References:

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CL was supported by a McNair Scholars Program Grant through GVSU.

474B Investigating the role of intrinsic protein disorder in transcription factor dynamics and function *Colleen Hannon*[1], Michael Eisen[1,2] 1) University of California, Berkeley, Berkeley, CA; 2) Howard Hughes Medical Institute, Berkeley, CA

Modern microscopy has revealed that core nuclear functions, including transcription, replication, and heterochromatin formation occur in spatially restricted clusters. Our lab is investigating the role that the sub-nuclear localization of transcription factors, target DNA sequences and transcriptional machinery plays in regulating RNA synthesis in the early *Drosophila* embryo. A nearly ubiquitous feature of eukaryotic transcription factors (TFs) is tracts of low complexity amino acid sequence, which result in intrinsically disordered regions (IDRs) within the protein. It has been proposed IDR-containing TFs mediate the co-localization of transcriptional machinery and target genes. However relatively little is known about the contribution of IDRs to TF localization, molecular interactions, and transcriptional activation. Using a novel algorithm to identify IDRs in the *Drosophila* proteome, we designed a library of IDRs from TFs expressed in the early *Drosophila* embryo. We used this library to conduct a broad survey of the nuclear sub-localization of TFs, using a high throughput imaging screen in *Drosophila* S2 cells. We found that while subnuclear clustering does not occur when the IDRs are expressed alone, it is frequently seen in full length TFs. These results are consistent in *Drosophila* embryos, suggesting that IDRs are insufficient to drive the sub-nuclear clustering behavior of transcription factors.

475C Nuclear Function of the protocadherin *fat* in *Drosophila* *Jannette Rusch*[1], Chikin Kuok[2], Joe Thanintorn[1], Yonit Tsatskis[2], Helen McNeill[1] 1) Washington University St Louis School of Medicine; 2) Lunenfeld-Tanenbaum Research Institute, Mount Sinai Hospital, Toronto, Canada

The conserved atypical cadherin *fat* (*ft*) controls cellular processes such as growth control via the Hippo pathway, planar cell polarity, and mitochondrial function, in organisms ranging from fruit flies to mammals. The intracellular domain of the Ft protein, FtICD, regulates a variety of partners to execute these functions. Our lab has found that FtICD is present in the nucleus in tissue culture cells, and we have identified both nuclear localization and nuclear export signals in Ft. Moreover, 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*. These observations suggested that *ft* may have a nuclear function in addition to its cellular signaling functions. ChIP experiments on *Drosophila* larval tissues identified putative *ft* targets, including the anti-apoptotic gene, *Diap1*, which is also a target of the Hippo pathway. Analysis of a small fragment of the *Diap1* enhancer, HRE (Hippo Response

Element), *in vivo* suggests that FtICD can activate this element, further supporting the novel notion of a nuclear function for *ft*. Remarkably, FtICD ChIPs to Yorkie sites across the genome. We hypothesize that the nuclear function of Ft functions to modulate Hippo pathway activity, complementing its established function as an upstream regulator of Hippo signaling.

476A Establishing the Role of the Conserved TN Domain in Tinman *Cayleen Bileckyj*, Richard Cripps San Diego State University

Congenital heart disease (CHD) is a major factor in mortality and morbidity in children and adults. Even though there has been substantial progress in detection and treatment, not all congenital heart defects can be identified early on through physical screenings. To advance our abilities to identify and manage CHD, we need to further understand the key genetic factors involved in causing these maladies.
Since *Drosophila melanogaster* shares similar cardiac developmental mechanisms with humans, it has been an essential model for human heart development. One similarity occurs between Tinman (Tin), a transcription factor in *Drosophila* necessary for the differentiation of cardiac cells, and its mammalian ortholog NK2 Homeobox 5 (Nkx2.5). These proteins share two conserved regions: the homeobox domain and the tin (TN) domain. Although the TN domain is completely conserved between these two proteins, there is little known about its significance.
By utilizing CRISPR/Cas9 gene editing, I established a line of *Drosophila* containing an in-frame deletion of the TN domain. Staining *Drosophila* embryos for NMR, a marker of cardial cells, revealed mutant embryos generate a significantly higher number of cardial cells when compared to wild type embryos ($p<0.01$). I also stained embryos for Tin and Svp, a protein important for heart development in *Drosophila*, to determine if the deletion of the TN domain affected cardial cell specification. Mutant embryos contained more cells expressing *tin* and more cells expressing *svp* than wild type embryos. These results support the importance of the TN domain in heart development and indicate the increase in cardial cells occurs before cardial cells differentiate into cells exclusively expressing *tin* or *svp*.
Tin and Nkx2.5 are vital to proper heart development. Characterizing the role of the conserved TN domain in these transcription factors provides the opportunity to vastly improve our understanding of the mechanisms involved in cardiac formation and maturation.

477B Initiating and Maintaining the Histone Locus Body: Two Sides of the Same Coin? *Greg Kimmerer*, Leila Rieder Emory University

Histone proteins play an essential role in nearly all genomic activities in eukaryotes, from gene regulation to DNA damage repair. In order to perform these functions, the ratio of DNA to histone levels must be kept constant, as deviations from this ratio cause cell cycle arrest and genomic instability. Consequently, a cell must double its histone content during each cell cycle. To meet these regulatory requirements, the histone genes are targeted by a conserved pantheon of chromatin proteins, known collectively as the Histone Locus Body (HLB). Most of the dozens of HLB components have no DNA-binding activity, yet they are able to faithfully locate the histone genes in the early embryo. The HLB must also be maintained at histone genes in adult tissues. How the HLB is initiated in the early embryo and maintained in the adult is a crucial gap in our knowledge of how proper histone gene regulation is achieved. Recently, our lab discovered that a single regulatory element located in the histone3/histone4 bidirectional promoter directs HLB initiation along the entire histone array. However, it is not clear if this cis-element, which consists of GA-dinucleotide repeats, is required to maintain the HLB after initiation. To answer this question, I have designed and cloned a novel transgene that takes advantage of the FLP/FRT recombination system in Drosophila. This transgene allows me to wait for the HLB to initiate in the embryo, then heat shock larvae to induce recombination, removing the GA-repeats. If the ectopic HLB is lost following heat shock, this will suggest that the GA-repeats plays an active role in maintaining the HLB. If the ectopic HLB survives heat shock, this will show that the HLB can be maintained even without the cis-element that initiated it, an equally illuminating result.

478C Developmental regulation of histone genes by pioneer factor Zelda *Thomas O'Haren*, Leila Rieder Emory University, Atlanta, GA

The zygotic genome is mostly unstructured and quiescent, so embryos are maternally loaded with proteins and RNAs that control early development. However, at some point, the zygotic genome must activate and begin transcribing necessary genes to assist in the early cellular divisions, a process known as zygotic genome activation (ZGA). The initial organization and transcriptional activation of the zygotic genome is facilitated by pioneering transcription factors that are able to bind and open regions of nucleosome-bound DNA. The histone proteins, which are critical to chromatin organization and regulation of the cell cycle, are some of the earliest expressed proteins from the zygotic genome. The five canonical, replication-dependent histone genes cluster in the genomes of metazoans, forming histone loci. A suite of factors known as the Histone Locus Body (HLB) act upon histone loci and regulate histone expression. The genome of *Drosophila melanogaster* carries a single histone locus of 107 tandem copies of the 5kb histone gene array. It remains unclear how the histone locus is activated and the HLB is established and maintained. The pioneer factor Zelda, a maternally deposited, zinc-finger protein, is the "master regulator" of ZGA and binds across the genome to "TAGteam"

sites, opening chromatin and marking surrounding regions for activation and transcription. We discovered the Zelda targets TAGteam sites in the histone locus as early as embryonic nuclear cycle 8. A histone array transgene in which the TAGteam sites are ablated fails to attract HLB factors suggesting that Zelda is necessary for HLB formation in the early embryo and is involved in activating zygotic histone gene expression. In the future, we will restore Zelda to the transgenic histone array using dCas9 and assay rescue of HLB formation. Overall, our results indicate that one indirect mechanism through which pioneer factors remodel the zygotic genome is through zygotic histone gene expression.

479A Fruitless modulates the threshold of Notch target gene transcription during asymmetric neuroblast division *Arjun Rajan*[1], Lucas Anhezini[1], Megan Neville[2], Elizabeth Larson[3], Stephen Goodwin[2], Melissa Harrison[3], Cheng-Yu Lee[1,4,5] 1) Life Sciences Institute, University of Michigan, Ann Arbor, MI; 2) Centre for Neural Circuits and Behaviour, University of Oxford, Oxford, UK; 3) Department of Biomolecular Chemistry, University of Wisconsin, Madison, WI; 4) Department of Cell and Developmental Biology, University of Michigan Medical School, Ann Arbor, MI, 48109 ; 5) Division of Molecular Medicine and Genetics, Department of Internal Medicine, University of Michigan Medical School, Ann Arbor, MI

A defined threshold of gene transcription prevents the accumulation of excess transcripts and proteins, allowing cells undergoing developmental transitions to rapidly rewire their gene regulatory circuits and assume new cell identities. The delay negative feedback loop that maintains the oscillatory pattern of Notch target gene expression in vertebrates exemplifies how post-transcriptional regulatory mechanisms function as a timer to indirectly regulate gene transcription levels. We investigated if repressive inputs could function in synchrony with transcriptional activators to modulate gene transcription thresholds in fly larval brain type II neuroblasts, which divide asymmetrically to generate a Notch^{ON} neuroblast and a Notch^{OFF} differentiating progeny. We hypothesize that factors eliciting repressive inputs should (1) be specifically expressed in type II neuroblasts, (2) bind Notch target gene loci and (3) repress Notch target gene expression. We performed single cell RNA-sequencing to establish a transcriptomic atlas of a wild-type type II neuroblast lineage that includes neuroblast progeny undergoing commitment to differentiation. We discovered that *fruitless (fru)* gene transcripts are uniquely detected in type II neuroblasts, and the C isoform of Fru (Fru^{c}) is specifically expressed in type II neuroblasts. Loss of *fru^{c}* function enhanced the supernumerary neuroblast phenotype in *numb*- or *brat*-hypomorphic brains in which reduced function of either gene leads to aberrant upregulation of Notch signaling in neuroblast progeny driving their reversion. We performed CUT&RUN to profile Fru^{c}-bound loci in type II neuroblast enriched brains and found that Fru^{c} binds most Notch target gene loci in the fly genome. These data suggest that Fru^{c} functions to antagonize Notch target gene expression in type II neuroblasts. Consistent with this model, reducing *fru^{c}* function increases Notch target gene expression levels in mitotic type II neuroblasts. Furthermore, overexpressing wild-type Fru^{c} or a constitutive repressor form of Fru^{c} is sufficient to downregulate Notch signaling and partially restore differentiation in *brat*-null brains. We propose that the balance between Fru^{c}-mediated chromatin modifications and Notch-induced histone acetylation specifies Notch target gene transcription thresholds in type II neuroblasts.

480B Out of the shadows: Co-acting *cis*-regulatory elements control T-box transcription factors *midline* and *H15* during development. *Cody Stevens*, Nir Yakoby Rutgers University Camden

Tissue development involves highly coordinated expression of transcription factors controlled by complex *cis*-regulatory elements (CREs). The posterior end of the follicular epithelium is patterned by Midline (MID), the *Drosophila* homolog of the mammalian Tbx-20 transcription factor. Here, we identify two CREs that recapitulate the endogenous pattern of *mid*. It has been genetically shown that *mid* is negatively regulated by the Iroquois transcription factor *mirror* and the bone morphogenetic protein signaling pathway, while positively activated by the ETS transcription factor *pointed*. In this study, we corroborate these *trans* regulators through *cis* regulation of *mid*, highlighting regulatory regions within the CREs. Furthermore, through CRISPR/Cas9 genome editing, we illustrate a redundancy of *mid* CREs. However, deletion of both CREs resulted in a significant reduction, but not loss of *mid*, indicating additional CREs are possible. Interestingly, unlike the published *trans* regulatory connection between MID and a second Tbx-20 homolog *H15*, we found co-regulation between one *mid* CRE and *H15,* whereas deletion of this CRE eliminated *H15* transcription. These results suggest that both T-box transcription factors *mid* and *H15* share regulatory capacity that may act as safeguards for determination of posterior fate during egg development.

481C Necessity versus sufficiency: furthering understanding of *ftz cis*-regulatory elements in *Drosophila melanogaster* *Matthew Fischer*, Patricia Graham, Leslie Pick University of Maryland, College Park

Spatiotemporal regulation of transcription is essential for directing complex patterns of gene expression during embryogenesis. This is achieved through the action of *cis*-regulatory elements (CREs), which have been studied extensively in *Drosophila* with reporter genes that permitted the *in vivo* dissection of CREs. How these CREs dynamically interact with each other and specific promoters in the context of native chromatin remain as unresolved questions. The *Drosophila Hox* complex includes the pair-rule gene *fushi tarazu* (*ftz*) nested between the homeotic genes *Antennapedia* and *Sex combs reduced* (*Scr*). Interestingly, *ftz and Scr* CREs are intermixed, but the genes have

distinct expression patterns: *ftz* is expressed in seven stripes during the blastoderm stage while *Scr* is expressed later in the embryonic labial and prothoracic segments. Our investigation is twofold; one, to assess the necessity of previously identified *ftz* CREs in the native context of the genome using CRISPR-Cas9 induced deletions; two, to identify the location of potential structural elements in this genomic region through the use of reporter transgenes. To this end, we independently deleted the zebra and upstream CREs, two classic seven-stripe *ftz* CREs, from the endogenous context of the *ftz* locus using CRISPR/Cas9. These deletion mutants are homozygous viable and fertile, contrary to expectations from historic rescue experiments. While some of these homozygotes develop all segments, other progeny develop abdominal abnormalities including specific segmental deletions and/or fusions. Quantification of the *ftz* signal using *in situ* hybridization chain reaction showed that *ftz* expression is reduced to 25% of wildtype in stripe four of the zebra deletion mutant. This refines the lower threshold of *ftz* transcript that is both necessary and sufficient for segmentation to proceed. Similar deletions have been made of several stripe-specific CREs, and these homozygotes are viable and fertile. Their survival demonstrates redundancy of multiple CREs directing *ftz* expression. Analysis of reporter transgenes generated in both orientations has identified two putative insulators. These regions will be targeted for deletion by CRISPR-Cas9 to determine if removing them causes crosstalk between *ftz* and *Scr*. Overall, this work provides insight into how insulators organize specific enhancer-promoter interactions in complex genomic regions, allowing for precise spatiotemporal regulation.

482A Defining the mechanisms underlying how enhancer binding sites regulate Notch signal strength *Collin Christensen*[1], Yi Kuang[2], Brian Gebelein[1, 2] 1) University of Cincinnati; 2) Cincinnati Children›s Hospital

Notch signaling regulates distinct cellular fates and processes during the development of nearly every organ in the body. Moreover, Notch-dependent processes are often highly sensitive to Notch signal strength as evidenced by genetic haploinsufficiency across multiple species. For example, decreases in Notch signal strength have been associated with developmental syndromes such as Alagille Syndrome and Adams Oliver Syndrome. However, we still do not fully understand how changes in Notch signal strength affect cell-specific responses. In general, Notch signal strength is determined by ligand induced production of the Notch Intracellular Domain (NICD), which forms a complex with the CSL transcription factor, Suppressor of Hairless (Su(H)) in flies, to induce gene expression via two types of binding sites: monomeric (CSL) and dimeric (Su(H) paired sites, SPS). Unexpectedly, we found that simply inserting synthetic enhancers that couple dimeric SPSs, but not monomeric CSL sites, with binding sites for the Grainyhead pioneer transcription factor resulted in flies that develop wing nicks, a classic Notch-sensitive phenotype. Moreover, this enhancer induced phenotype is highly sensitive to genetic changes in both the Notch pathway and the Cdk8-Mediator complex, which has been previously shown to promote NICD turnover. These findings suggest that Notch dimer sites preferentially promote NICD turnover and that Grainyhead can aid this process by promoting chromatin accessibility. To test this model, we generated a series of enhancers that couple SPS sites with binding sites for other pioneer transcription factors or transcription factors not known to have pioneer activity. Importantly, we found that pioneer factors, such as Zelda and Trithorax-like, could produce wing nicks when placed next to SPS sites, whereas non-pioneer sites such as E-box and UAS sites failed to do so. Our results also revealed that some, but not all, endogenous dimeric SPSs produced wing nicks, suggesting that other factors influence this activity. Genetic dosage experiments in *Drosophila* further revealed that removing a copy of each of the Cdk8-Mediator components significantly reduced wing nicking. Ongoing studies are focused on testing whether Grainyhead promotes the opening of these synthetic enhancers and on identifying the additional sequences and factors that allow for a subset of SPSs to induce wing nicking phenotypes.

483B *brinker* gene promoter-proximal element drives ovary expression and supports sequential action of distal enhancers *Susan Newcomb*, Leslie Dunipace, Angelike Stathopoulos California Institute of Technology (Caltech)

Limiting BMP signaling range in the stem cell niche of the ovary protects against germ cell tumors and supports germline homeostasis. In both the embryo and wing disc, the Brinker (Brk) transcription factor serves as the canonical repressor of Dpp, the main *Drosophila* BMP ligand, and its downstream targets. In the germline stem cell niche within the germarium of the ovary, Dpp provides a pivotal cue for maintaining stem cell identity. Despite this well-established role, Brk's function in the germarium has not previously been described. Here we find that *brk* expression throughout the ovary requires a promoter-proximal element (PPE), a DNA region upstream of and distinct from the gene promoter. In the germarium, the PPE acts as an enhancer to regulate *brk* expression levels to support germline stem cell homeostasis and, surprisingly, to positively regulate Brk's canonical antagonist, *dpp*. This PPE has been previously described in the embryo where it does not itself drive expression, but is required for the sequential action of two distal enhancers to support continuous brk expression. In the egg chambers of the ovariole, at least two enhancers perform a similar temporal handoff and their activities require the PPE, indicating that this element may serve a general function of managing gene regulation by multiple enhancers over time by coordinating shifts in local chromatin conformation.

484C Investigating the genome-wide cooperativity between the pioneer factor Zelda and patterning transcription factors in the early embryo *Kaelan Brennan*[1], Melanie Weilert[1], Sabrina Krueger[1], Julia Zeitlinger[1,2] 1) Stowers Institute for Medical Research, Kansas City, MO; 2) The University of Kansas Medical Center, Kansas City, KS

Enhancers, or cis-regulatory DNA sequences that control spatiotemporal gene expression programs, have intrinsically high nucleosome barriers that prevent the nonspecific binding of transcription factors (TFs). For an enhancer to become active, TFs must overcome this barrier, access their binding motifs, and evict the nucleosome, but the mechanisms by which these steps occur are not clear. Current models suggest that specialized TFs called pioneer factors can access their motifs in the presence of nucleosomes and foment nucleosome depletion through cooperativity with additional TFs. Even still, how pioneer and non-pioneer TFs cooperate to generate chromatin accessibility at enhancers is not yet known. To understand how TFs overcome the nucleosome barrier, we are using Zelda, a pioneer TF for the *Drosophila* maternal-to-zygotic transition, and a set of TFs that drive pattern formation in the early embryo (patterning TFs) as a model system. To dissect Zelda's cooperativity with the patterning TFs, we have integrated ChIP-nexus TF binding data with our novel deep learning model, BPNet, which learns the relationship between DNA sequence and TF binding in an inherently combinatorial way. BPNet reliably predicts the genome-wide binding of Zelda and the patterning TFs, and successfully discovers known and novel motifs at well-studied enhancers. Through employing the trained BPNet model as an *in silico* oracle, we reveal that Zelda and the patterning TFs exert directional cooperativity within nucleosome-range distances. Using time course ATAC-seq measurements, we find evidence that Zelda alone is insufficient to generate accessibility, rather chromatin accessibility in the early embryo relies on cooperativity between Zelda and the patterning TFs. Taken together, these results suggest a nucleosomal basis for cooperativity between Zelda and the patterning TFs and present a model where Zelda establishes a more permissive chromatin state for TF binding but relies on cooperative interactions with the patterning TFs to bestow accessibility. To further dissect the hierarchical nature of TF cooperativity for chromatin accessibility, we are training a BPNet model on our time course ATAC-seq data, which will learn the relationship between DNA sequence and accessibility and will identify the sequence features that are predictive of chromatin accessibility.

485A Enhancer hijacking leads to flies with no thorax *Taylor Crawford*, Anna Horacek, Victoria Blake, Judith Kassis NICHD/NIH

The *invected* (*inv*) and *engrailed* (*en*) genes are expressed throughout development in *Drosophila melanogaster*. Distinct enhancers drive the co-expression of *inv* and *en* in discrete parts of the embryos including in stripes, parts of the head, and the CNS. Inv/En are also co-expressed in the anterior/posterior compartment of larval imaginal discs. The *inv* and *en* promoters are separated by ~54 kb and their expression is regulated by enhancers distributed across a ~100 kb region. One enhancer that regulates expression in imaginal discs is located 40kb and 90kb upstream of the *en* and *inv* promoters. How does this imaginal disc enhancer find the *en/inv* promoters? Previous results from our lab suggested that there is a promoter-proximal tethering element (PTE) that facilitates this activation. We are interested in understanding how this PTE functions. Our lab isolated a transgenic line containing a 2 kb *en* regulatory fragment, including the PTE, fused to a *Beta-galactosidase* reporter gene, in a P-element vector including the mini-white reporter gene, inserted 250bp upstream of the endogenous *en* promoter. Flies heterozygous for the transgene show expression of *Beta-galactosidase* and *en* in the posterior compartment of the wing imaginal discs. However, homozygotes show a reduction in *en* expression in wing imaginal and die as pharate adults with no thorax. These results indicate that the imaginal disc enhancers are hijacked by the transgene, causing a tissue-specific loss-of-function phenotype. We are investigating which sequences are required to mediate enhancer hijacking by the transgene using the Cre-loxP recombination system to insert various transgenes 250bp upstream of the *en* promoter. We have identified a 181bp DNA fragment that, when combined with a minimal promoter, captures imaginal disc enhancers and disrupts *en* expression. Interestingly, this fragment also acts as a Polycomb response element (PRE) in which it can recruit Polycomb group proteins. In addition to identifying sequences necessary for enhancer-promoter communication, our study shows that interfering with promoter-enhancer communication is another way to generate a tissue-specific mutant phenotype.

486B Investigating the role of Notch signalling in the development of the ventral mesoderm in *Drosophila melanogaster* *Marvel Megaly*, Gregory Foran, Aleksander Necakov Brock University

Notch signaling is a critical regulator of multiple developmental processes through its ability to control gene expression, and thereby influence cell fate specification and cell proliferation through direct cell-cell communication. Notch signaling is activated through binding of the transmembrane ligand, Delta, to the Notch extracellular domain (NECD) of the transmembrane Notch receptor. Ligand engagement and trans-endocytosis into the signal-sending cell drives removal of the labile NECD, and concomitant cleavage of the Notch intracellular domain (NICD). Upon subsequent translocation to the nucleus, the NICD regulates target gene expression through its interaction with Suppressor of Hairless Su(H). Although Notch signaling has been shown to play a role in regulating *single minded (Sim*) expression in the embryonic mesectoderm, Notch activity and function in the directly-adjacent cells of the ventral mesoderm remain unknown. Considering that Delta endocytosis and NECD trans-endocytosis are required for Notch signal activation, and are restricted to the ventral mesoderm in the early embryo, we investigated the role of Notch signaling in the ventral mesoderm. To test our hypothesis, we have used a combination of Optogenetics, quantitative RNA fluorescent *in situ* hybridization (FISH), and qPCR. Through *in silico* analysis, we identified 13 potential Notch target genes based on the following three criteria: 1) expression in the embryonic ventral mesoderm, 2) promoter-resident Su(H) binding site(s),

and 3) differential expression during ventral mesoderm formation. We have validated these candidate Notch target genes, which include *WntD, Asph, Heartless, Traf4, Tinman, Stumps, Mef2, Mes2, mir-1, Neurotactin, and NetrinA,* by comparing the level of gene expression between loss-of-function Notch mutants and wild-type embryos through two orthogonal techniques; FISH and qPCR. Consistent with a role for Notch signaling, expression of these mesoderm-specific genes is reliant upon Notch. These results prompted us to ask whether Notch signaling is sufficient to drive expression of these mesodermal target genes. To address this, we have developed and validated a set of novel Optogenetic tools to ectopically activate Notch signaling in a precise spatio-temporal manner. Consistent with previous findings, we observed that ectopic activation of Notch signaling is sufficient to drive *Sim*, a Notch target gene normally restricted to the mesectoderm. Interestingly, we have observed a stage-dependent expansion of *Sim e*xpression into both the ectoderm and the ventral mesoderm of the early embryo, along with a change in the position of the ventral mesodermal boundary, demonstrating an effect of precocious Notch activity on embryonic patterning.

487C The synergistic roles of Glass and EGFR signaling in the differentiation of multiple retinal cell types *Hongsu Wang*[1], Raja Komal[2], Kelvin Yeung[2], Graeme Mardon[2], Jessica Treisman[1] 1) Skirball Institute for Biomolecular Medicine and Department of Cell Biology, NYU School of Medicine; 2) Department of Pathology and Immunology and Department of Molecular and Human Genetics, Baylor College of Medicine

Light-detecting photoreceptors and supportive cone cells differentiate from identical precursor cells in the eye disc during late larval and early pupal stages. Reiterative Epidermal Growth Factor Receptor (EGFR) signaling induces the specification of photoreceptors R1-7 and cone cells. Glass (Gl) is an eye-specific transcription factor that is necessary for the differentiation of all cell types in the retina. Gl is expressed in all cells posterior to the morphogenetic furrow, but only turns on photoreceptor-specific genes in cells that receive an EGFR signal. We hypothesized that the cell-intrinsic transcription factor Gl and the external EGFR signal synergistically induce accurate and robust differentiation. Misexpression of Gl in the wing disc leads to the activation of some eye-specific genes, but only coexpression of Gl with an activated form of the EGFR signaling component Ras (Ras^{v12}) could induce expression of the neuronal markers Elav and Futsch. We used RNA-Seq to identify genes that are synergistically induced by Gl and Ras^{V12} in the wing disc and to determine which of them are dependent on the presence of the transcription factor Pointed (Pnt), which acts downstream of Ras. A large proportion of these Pnt-dependent synergistically induced genes are characteristic of neurons. We are using Targeted Dam ID with Gl and PntP1 to determine which of these genes are direct targets of Gl and/or EGFR signaling. Alternatively, transcription factors that are regulated by the combination of Gl and Ras^{V12} could mediate their downstream effects, and we are testing several candidates. Single-cell RNA-Seq revealed that *gl* mutant eye discs lack both the distinctive differentiation trajectory profile of photoreceptors R2, 5, 3, and 4 present in wild type white prepupal eye discs (accuracy of differentiation) and any clusters representing R1, 6, or 7 (robustness of differentiation). Studying the synergy between Gl and EGFR signaling will help us understand how the cell intrinsic factor Gl cooperates with external signaling to induce accurate and robust differentiation of key retinal cell types.

488A Tissue-specific diversity of the *Muscleblind* expression in adult flies Davron Hanley Kennesaw State University

The *muscleblind* (*mbl*) family of RNA-binding proteins regulates alternative splicing, determining mRNA transcript composition for various types of tissue, and has been implicated in myotonic dystrophy. The *mbl* gene is subject to alternative splicing in *Drosophila*, leading to multiple isoforms, and has several paralogs in humans. Mbl proteins vary significantly in length, although the significance of such diversity and the role of specific isoforms have not been fully explored.
Using immunofluorescence microscopy and polyclonal serum, we analyzed Mbl protein expression across adult *Drosophila* tissues. Mbl was detected in various locations, including the brain, gonads, muscle, and gut epithelium. Skeletal muscles demonstrated the greatest diversity in Mbl expression, with other tissues showing more homogenous expression. Mbl was present at low levels in flight and jump muscles, while other thoracic muscles and abdominal muscles showed high Mbl levels. Intracellular localization of Mbl was typically nuclear, however in the nervous system the protein was strongly expressed in the cytoplasm. During early adult development in the pupa, in various tissues, Mbl was initially detected in discrete nuclear bodies, before it assumed more general nuclear staining.
Our study reveals natural locations that have drastically different levels of Mbl as well as its intracellular localization. Our further aim is to supplement these findings with molecular analysis of Mbl isoforms to create the basis for functional interrogation of the Mbl diversity and its relation to tissue-specific regulation of alternative RNA splicing.

489B Lingerer interact with FMRP to promote FMRP target translation *KAICHENG MA*, Al Rohet Hossain, Kayla Judson, Ethan Greenblatt The University of British Columbia

Fmr1 encodes an RNA binding protein FMRP necessary for proper ovary and neuronal development. Mutations in Fmr1 lead to fragile X syndrome, the most commonly inherited intellectual disability (ID) autism-associated disorder. By studying Fmr1's role in transcriptionally silent mature Drosophila oocytes, we recently discovered that Fmr1 functions primarily to promote the translation initiation of genes encoding large proteins, many of which are orthologs of human genes associated with ID and autism.

In order to identify FMRP binding partners and elucidate the mechanism of Fmr1-dependent translation, we used CRISPR to tag Fmr1 at its endogenous locus and performed IP-mass spectrometry experiments from ovarian extracts. We also determined which FMRP binding partners are required for its activity by using a novel single molecule fluorescence in situ hybridization (smFISH)-based assay, in order to assess Fmr1's activity in the absence of its binding partners.

Our preliminary data identify a critical role for the RNA and ubiquitin binding protein lingerer (lig). We find that lig physically associates with FMRP, and the loss of lig appears to strongly diminishes FMRP's activity towards one of its targets. Previous studies of the mammalian lig ortholog UBAP2L in cultured cells have shown that it can bind to ribosomes to promote translation. In flies, lig is required for proper copulation behavior and short-term memory. Our data indicate that lig acts in vivo with FMRP to promote the production of FMRP targets, a function which is likely to be critical for proper neuronal and ovarian function.

490C Development of a novel molecular assay to sensitively detect Fmr1's translational function in *Drosophila* ovarian follicles. *Kayla Judson*, Al Rohet Hossain, Kaicheng Ma Department of Biochemistry and Molecular Biology, UBC, Vancouver, BC

Loss of function mutations in the Fragile X Mental Retardation 1 (Fmr1) gene are associated with fragile X syndrome and fragile X-associated primary ovarian insufficiency, leading causes of autism and female infertility respectively. Although Fmr1 has been highly studied, its biological role is still subject to debate. A major challenge in studying Fmr1 is that its molecular function is to weakly promote the translation of its targets ~2-fold, making it difficult to assess its activity *in vivo*. Here we present a novel assay to detect Fmr1's function at a molecular level with high sensitivity in developing *Drosophila* ovarian follicles.

Using single molecule fluorescence *in situ* hybridization (smFISH), we observed that the mRNA of the Fmr1 target Poe forms microscopically visible granules that depend on ongoing translation. These "Poe particles" appear to be sites of Fmr1 translational activation, as Fmr1, Poe mRNA, and fully translated Poe protein are each enriched in Poe granules. Fmr knockdown, or treatments that reduce translation (puromycin or heat shock) resulted in a robust dispersal in Poe mRNA granules. Using the presence of Poe particles as a sensitive readout of Fmr1 activity, we conducted a genetic screen to identify potential partners of Fmr1, by labeling Poe mRNAs in either wild type egg chambers or those lacking Fmr1 or candidate genes. We screened over a hundred different candidate genes using germline-specific RNAi. We found that only a few RNAi lines targeting RNA binding proteins and/or proteins which physically bind to Fmr1 exhibited a strong reduction in Poe particles. Our findings suggest that Fmr1 functions together with a small set of additional factors to promote the expression of genes essential for normal ovarian and neuronal function.

491A Precocious expression of Zelda does not initiate early zygotic genome activation *Elizabeth Larson*[1], Zoe Fitzpatrick[1], Hideyuki Komori[2], Cheng-Yu Lee[2], Melissa Harrison[1] 1) Department of Biomolecular Chemistry, University of Wisconsin School of Medicine and Public Health, Madison, WI; 2) Department of Cell and Developmental Biology and Life Sciences Institute, University of Michigan, Ann Arbor, MI

During the first stages of development, the fertilized germ cells rapidly transition to totipotency. Maternally deposited mRNAs encode the proteins necessary for reprogramming the transcriptionally quiescent zygotic genome during this maternal-to-zygotic transition (MZT). The transcription factor Zelda is essential for this reprogramming in the *Drosophila* embryo. Zelda is necessary for transcriptional activation of the zygotic genome, and the absence of Zelda leads to embryonic lethality during the MZT. Excess Zelda activity is also lethal to the embryo, demonstrating that Zelda levels must be precisely controlled during early development. Because Zelda is encoded by a maternally deposited mRNA, Zelda levels in the embryo are controlled at the level of translation. To understand how levels of this essential reprogramming factor were regulated to allow for embryonic development and zygotic genome activation, we investigated the factors that regulated translation of *zelda*. Brain Tumor (BRAT) is a translational regulator that was previously shown to bind to *zelda* mRNA in the embryo. We showed that BRAT functions to repress *zelda* translation, as embryos deficient for maternal BRAT activity prematurely express Zelda. We further showed that in the larval brain, BRAT similarly regulates Zelda levels and identified specific BRAT-binding sites that mediate these effects. Thus, BRAT regulates Zelda in multiple tissues. Because both too little and too much Zelda are lethal to the embryo, we hypothesized that precocious expression of this transcriptional activator might be capable of driving precocious activation of the zygotic genome, leading to embryonic lethality. To test this hypothesis, we performed single embryo RNA-seq at distinct nuclear cycles throughout zygotic genome activation (NC10, NC12, NC13, and NC14) in control and *brat*-mutant embryos. Our results conclusively demonstrated that in embryos lacking functional BRAT, Zelda target genes were not prematurely

activated. Rather, these genes were expressed normally, but become significantly upregulated at nuclear cycle 14, when the division cycle slows. Our data support a model in which zygotic genome activation requires precise coordination between expression of reprogramming factors, such as Zelda, and the slowing of the cell cycle.

492B Tet (Ten-Eleven Translocation) Regulates Axonal Development in the *Drosophila* Pupal Brain via Transcriptional Repression *Hiep Tran*, Badri Nath Singh, Joseph Kramer, Ruth Steward Waksman Institute, Rutgers University

Tet (Ten-eleven translocation) is one of the most important epigenetic regulators. In vertebrates, TET1/2/3 control demethylation DNA to activate gene transcription. Both vertebrates and *Drosophila* Tet can also hydroxymethylate cytosine in mRNA (5hmrC). Our recent results show that *Drosophila* Tet is essential for viability and that the protein plays crucial roles in brain and muscle development. Further Tet binds to 2,500 genes and appears to deposit the 5hmrC modification to their mRNAs thereby enhancing their translation levels. In this study, we found that Tet is required for mushroom body axon outgrowth in the pupal brain. Lack of Tet resulted in β lobe axon crossing the brain midline. By comparing CRISPR/Cas9-induced point mutations in the Tet DNA binding domain (Tet^{AXXC}) and catalytic domains (Tet^{YRA}), we discovered that in the DNA binding domain mutant Tet^{AXXC} the β lobe axons crossed the brain midline. This phenotype was not observed in the catalytic domain mutant Tet^{YRA}. Surprisingly, in the DNA binding domain mutant, the expression of 1,597 genes in developing pupal brains were increased while the catalytic domain mutant only showed 28 genes up- and 16 genes down-regulated. Comparing these results with Tet ChIP-seq data from our recent study shows that 448 Tet-bound genes are upregulated in the DNA binding domain mutant. The RNA of 444 out of 448 genes do not show 5hmrC modification, indicating a distinct function of the Tet DNA binding and catalytic domains. The genes whose RNA level is increased in Tet^{AXXC} exhibit low levels or the absence of expression in wild-type brains demonstrating that Tet can suppress gene transcription via its DNA binding domain. Our study depicts a new function of Tet protein; it regulates axon outgrowth via an epigenetic mechanism in which Tet binds DNA and suppresses unwanted gene transcription in the brain, independent of cytosine methylation.

493C (E)close but no cigar: Essential developmental programs transcriptionally regulated by the chromatin modifier KDM5 *Michael Rogers*[1], Coralie Drelon[1], Helen Belalcazar[1], Owen Marshall[2], Julie Secombe[1] 1) Albert Einstein College of Medicine, Bronx, NY; 2) Menzies Institute for Medical Research, University of Tasmania, Hobart, Australia

Chromatin-regulating proteins are powerful supervisors of the genome that are critical for the precise control of gene expression. Developmental processes depend on these proteins for the coordinated regulation of transcriptional programs that are essential for proper tissue formation and organismal maturation. However, there still exists significant gaps in knowledge about the mechanisms of this fine-tuning aspect of gene expression, and tissues like the prothoracic gland provide fantastic settings to dissect these dynamics during development. Here we show that lysine demethylase 5 (KDM5, formerly Lid), a histone modifier capable of dynamically regulating chromatin through multiple functions, plays critical roles in the prothoracic gland. Interestingly, animals lacking canonical KDM5 histone demethylase activity are viable, but *kdm5* null mutants exhibit delayed development and a failure to eclose after metamorphosis. Although KDM5 is ubiquitously expressed across the organism throughout development, we have demonstrated that restoring KDM5 expression specifically within the prothoracic gland of *kdm5* null mutant animals rescues both the larval developmental delay and pupal lethality. Our studies show that KDM5 regulates MAPK signaling within prothoracic gland cells that functions in promoting endoreplication and ecdysone hormone production. Leveraging Targeted DamID technology, we have generated the first genomic-binding data set (akin to ChIP-seq) for prothoracic gland cells and identified the genomic targets of KDM5 localization across the genome. We are currently integrating these data with transcriptome, interactome, and cellular analyses to characterize KDM5-mediated mechanisms of gene expression regulation in these cells. 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 coordinate development.

494A Extracellular neuronal stimulation promotes Tip60 histone acetyltransferase mediated epigenetic neuroplasticity gene control in the *Drosophila* brain. *Christina Thomas*, Ellen Armour , Felice Elefant Drexel University

Experience-dependent (ED) plasticity is central for establishing memories and can influence the severity of AD progression. In this regard, we demonstrated that extracellular stimulation of rat hippocampal neurons *in vitro* triggers epigenetic gene regulator Tip60 nuclear import with concomitant genomic reorganization and synaptic gene induction. We further show that Tip60 nuclear import is recapitulated in the fly brain *in vivo* in response to stimulating neurons inducibly or by exposure to ED living conditions. Strikingly, Tip60 is found excluded from hippocampal nuclei in AD patients. Here we test the hypothesis that Tip60 extracellular mediated subcellular dynamics functionally impact synaptic gene control and cognition *in vivo* and that this process goes awry during early stages of AD. Using genetically modified *Drosphila* to inhibit the *Shaker* potassium pump within the neuronal membrane, we induced neuronal stimulation in the fly brain. We then used qPCR on RNA isolated from dissected brains in induced versus non-induced flies to assess expression levels of activity dependent plasticity genes we previously demonstrated to be *bona-fide* Tip60 targets. Strikingly, we found that as Tip60 increased within the nucleus, expression of specific Tip60 target neuroplasticity

genes including dlg, dsh, and futsch were induced. These results suggest that Tip60 shuttles into the nucleus *in vivo* to activate gene expression of synaptic plasticity genes. Future directions will focus on exploring the colocalization of Tip60 with transcription factors (TFs) to further elucidate the mechanism underlying Tip60's role in activity dependent gene control and ED plasticity.

495B Extracellular stimulation triggers Tip60 HAT nucleocytoplasmic transport in the *Drosophila* brain with concomitant induction of Tip60 target neuroplasticity genes *Ellen Armour*, Gabrielle Greco, Felicity Khoa, Felice Elefant Drexel University

Experience-dependent (ED) plasticity is central for establishing memories and is driven by epigenetic mechanisms that regulate dynamic gene transcription in response to neuronal stimulation. Our lab has previously shown the histone acetyltransferase (HAT) Tip60 shuttles into the nucleus *in vitro* to regulate activity dependent gene control of synaptic plasticity genes. Furthermore, we also found that Tip60 is largely excluded from the nucleus in hippocampal neurons from Alzheimer's disease (AD) patients. However, if and how Tip60 shuttles in the *in vivo* brain to regulate the expression of activity dependent synaptic genes is yet to be elucidated. Here, we used immunohistochemistry (IHC) to compare the subcellular localization of Tip60 HAT in the *Drosophila* brain under normal conditions and in response to stimulation of fly brain neurons *in vivo* either by inducibly activating potassium channels using genetic manipulation or by exposure to natural ED living conditions. Strikingly, we found that both inducible and ED living condition mediated neural induction triggered changes in Tip60 subcellular localization evidenced by a significant increase of Tip60 in the nucleus with concomitant induction of previously identified Tip60 target genes. Our results support a model by which neuronal stimulation promotes nuclear import of Tip60 to promote induction of activity-dependent neuroplasticity gene transcription. Future work will focus on labeling Tip60 with a GFP tag to track its *in vivo* nucleocytoplasmic transport in real-time and determine whether this processes is disrupted in brains from *Drosophila* that model AD. We will also mutate the nuclear localization sequences and nuclear export sequences to determine the functional impact of disrupting Tip60 nucleocytoplasmic transport on cognition and use chromatin assays to explore underlying mechanisms.

496C Reguation of Polycomb silencing initiation during nurse cell development Steven DeLuca Brandeis University

Polycomb group (PcG) proteins repress transcription and maintain a molecular memory of gene inactivity that is important for embryonic patterning and cell differentiation. Where and when Polycomb silencing initiates is therefore critical for producing correct gene expression patterns during later development. While biochemical and genetic approaches have identified many PcG proteins, how these proteins collaborate to correctly initiate silencing in genomic space and developmental time and poorly understood. We developed a new model system to study Polycomb silencing initiation. We recently discovered that nurse cells in the Drosophila ovary initiate Polycomb silencing on common target genes similarly to somatic cells in the early embryo. During silencing initiation, accessory proteins remodel PRC2 mobility across the transcriptionally inactive B-compartment. Faster migrating PRC2 complexes methylate and silence most inactive regions and slower migrating complexes focus PRC2 activity on traditional Polycomb target genes. By combining genetics and live imaging, we determined how individual PcG proteins contribute to this transition. A single protein, Scm, is critical for establishing high concentrations of PcG proteins on target genes during the initiation step. We will present our recent findings for how Scm is developmentally regulated and how it contributes to the site-specificity of PcG protein targeting. We will also present our ongoing efforts to use single molecule tracking to measure the kinetic properties of PcG protein complexes before and after silencing initiation.

497A Developmental ethanol exposure causes changes in the expression of histone modifying enzymes and results in long-term changes in gene expression *Joshua Marsh*, Madeleine Mok, Rachael French 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 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 tested 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*, and *NO66* result in changes in sensitivity to DAE. Additionally, DAE causes reduced expression of *G9a*, *Sirt1*, and *Lid* in larvae. 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. In addition, we are testing whether persistent DAE-induced phenotypes are due to changes in epigenetic regulation of gene expression.

498B Epigenetic regulation of energy homeostasis by the RNA adenosine methylation Daniel Wilinski[1], *Tahrim Choudhury*[1,2], Monica Dus[1] 1) Department of Molecular, Cellular and Developmental Biology, The University of Michigan, Ann Arbor; 2) PREP Program, The University of Michigan, Ann Arbor

Epigenetic mechanisms control the proper function of beta cells and alterations in these pathways have been linked to the development of type 2 diabetes (T2D), a debilitating disease that affects ~30% of Americans and that has no cure. Among these epigenetic mechanisms, the methylation of N6-adenosines in mRNAs (m^6A) –which controls mRNA stability, turnover, and translation– has recently emerged as a novel regulator of ß-cell biology. In mammals, many of the mRNAs important for insulin secretion and signaling, including the insulin mRNA, are marked with this epigenetic mark and many are hypomethylated in the ß-cells of people with T2D. Consistent with this, mutations in the methyltransferase enzymes impair insulin secretion and glucose homeostasis in mammals, suggesting that m^6A directs essential aspects of ß-cells physiology. However, the molecular underpinnings of these phenotypes are not known and the precise function of this epigenetic pathway in the biology of the ß-cells remains largely a mystery. This lack of knowledge has hindered progress in uncovering the underlying causes of T2D and in developing new therapeutic interventions based on this epigenetic pathway. Here we propose to exploit the unique advantages of the fly *D. melanogaster* model to identify the molecular mechanisms through which the m^6A pathway regulates the physiology of ß-cells. We discovered that in flies mutations in the m^6A pathway give rise to similar cellular and physiological phenotypes measured in mammals. Our hypothesis is that this epigenetic pathway regulates the responses of the insulin cells to glucose and amino acids and the release of insulin. We are currently taking a multidisciplinary approach that includes molecular biology, genomics, *ex vivo* imaging, and metabolic measurements to test these hypotheses and will present the result of these experiments at the conference. Discovering how specific epigenetic mechanisms direct distinct ß-cells processes is relevant to public health because it will advance our understanding of the biology of these cells and the etiology of T2D.

499C Pleiotropic fitness effects at the *Uhg4-Boot* locus in *Drosophila melanogaster* *Rebecca A MacPherson*[1,2], Robert R. H. Anholt[1,2], Trudy F. C. Mackay[1,2] 1) Center for Human Genetics, Clemson University, Greenwood, SC; 2) Department of Genetics and Biochemistry, Clemson University, Greenwood, SC

U snoRNA host gene 4 (Uhg4) encodes an antisense long noncoding RNA that is moderately expressed throughout the fly, with highest expression in embryos and adult ovaries. *Uhg4* is host to seven small nucleolar RNAs (snoRNAs) and is coregulated with a subset of 37 snoRNAs that are differentially regulated in a background-dependent manner in females in response to developmental ethanol exposure. *Bootlegger* (*Boot*) is immediately upstream of and in opposite orientation to *Uhg4* and encodes a protein critical for nuclear export of noncoding piwi-interacting RNAs. Expression of *Boot* is almost exclusively confined to ovaries. We have generated multiple deletions spanning the first exon of *Uhg4* and the promotor regions of both *Boot* and *Uhg4* using CRISPR-*Cas9* and revealed a spectrum of pleiotropic effects on fitness traits, including increased development time, changes to sleep and stress response, background-dependent decreases in viability, as well as sterility in both sexes. RNA sequencing data suggest that the region between *Uhg4* and *Boot* is required for normal expression of *Uhg4,* two intronic snoRNAs hosted by *Uhg4*, as well as *Boot*, leading to 668 differentially expressed genes. Gene ontology enrichment analysis showed enrichment of genes involved in immune response, ribosome formation, and DNA repair. We compared CRISPR-*Cas9* deletion mutants of the *Boot-Uhg4* regulatory region with RNAi-mediated knockdown of *Boot* and found that many of the effects on organismal phenotypes can be attributed both to *Boot* and the lncRNA *Uhg4.* However, we also observe differences in sexual dimorphism as well as antagonistic effects on fitness traits due to disruption of these genes. Thus, this regulatory region in the Drosophila genome highlights a long noncoding RNA and a protein coding gene that are both essential for reproduction and fitness.

500A Using Natural Variation and Machine Learning to map Gene Regulatory Networks *Prasad Bandodkar*[1], Elle Rooney[2], Samiul Haque[2], Cranos Williams[2], Gregory Reeves[1] 1) Texas A&M University; 2) North Carolina State University

In most well-characterized Gene Regulatory Networks (GRNs), maps of GRNs remain incomplete. The GRN for regulating anterior-posterior (AP) patterning in early *Drosophila* embryos, is a well-studied network and most of the major components have been identified. However, the minor components of the network, responsible for compensatory

regulation, remain elusive. Typical molecular techniques, such as a knockout, could result in several lost connections, swamping out compensatory regulation. Instead, we are using natural variation in the ~200 fly lines in the Drosophila Genetic Reference Panel (DGRP) to identify and characterize novel components of the network, using machine learning techniques.

The segmentation gene network that patterns the anterior-posterior axis in the early embryo is hierarchical and we are investigating the time period that is roughly mid-way between the start of nuclear cycle 14 and cellularization. During this time period, maternal coordinate genes such as Bicoid (Bcd), activate and regulate Gap genes such as Krüppel (Kr), and together they regulate pair-rule genes such as even-skipped (eve). The idea is to quantify the spatial expression patterns of the genes of interest to the segmentation GRN and use the subtle differences in spatial positions to map the cause back to specific regions in the genome and to the transcriptome. With 13 fly lines and imaging two genes- Kr and eve, searching within a 20 kb region of the genome, we were able to identify *pangolin* (*pan*) as a novel component of the AP patterning network. With data from ~200 fly-lines, we expect to have enough statistical power to identify several new components. We have built image analysis pipelines to reliably extract spatial gene expression data from a large number of stained embryos without manual supervision. Using unsupervised machine learning techniques over dataset from all ~200 fly lines and searching across the genome and the transcriptome, we expect to uncover novel components of the AP patterning network, or at the very least better characterize the existing network.

501B Sources of variation in gene expression *Siddhant Kalra*, Stephen Lanno, Lupita Sanchez, Joseph Coolon Wesleyan University, Middletown, CT

A century of genetic research has revealed that there are numerous causes of organismal trait variation. These include important contributions from an organism's genotype, environment, age, and even the environment experienced by previous generations referred to as transgenerational effects. While a multitude of studies has demonstrated diverse and abundant effects of each source of variation in a wide variety of organisms, the relative contribution of the different sources of variation remains largely unknown. This has largely resulted from a lack of studies that simultaneously determine the contributions of each source of variation within a single experiment, and this is especially true for transgenerational effects that are in general poorly understood. Here we quantified genome-wide gene expression traits in *Drosophila* and compared the contribution of genotype (*D. simulans* vs. *D. sechellia*), environment (control food vs octanoic acid food), age/developmental stage (L3 larvae vs adult), and previous generation environment (control food vs octanoic acid food) to variation in gene expression levels. We followed this with analysis of genome-wide allele-specific expression in F1 hybrids between *D. simulans* and *D. sechellia* to disentangle the *cis* and *trans*-regulatory contributions to differences in variation for each source of variation. We found that genotype and developmental stage have much greater effects on gene expression than environmental differences and all were much more abundant than the variation due to trans-generational effects from previous generation exposure to different environments. By simultaneously analyzing major sources of variation in the same experiment, our results suggest a hierarchy of importance of the different sources of variation in traits that answers a long-standing question in genetics.

502C Using ISRES+, an evolutionary optimization algorithm to fit experimental data in systems biology models *Razeen Shaikh*, Prasad Bandodkar, Gregory Reeves Texas A&M University

Modern genetic research focuses on elucidating protein function and pathways. These involve interactions among various entities that influence gene regulation and expression. The field of Systems Biology seeks to devise experimental and computational approaches to understand how these interactions impact the behavior of the whole. However, systems biology models contain many unknown parameters and assumptions, and to improve their accuracy they must be fit to experimental data. One historically successful approach to fit data is to use evolutionary algorithms. These algorithms begin with randomly-selected parameter sets, which are improved in each generation by ranking and selecting the best parameter sets. These parameter sets are then adapted to experimental data using mathematical strategies like "recombination" and "mutation" to reach a global optimum.
Improved Stochastic Ranking Evolution Strategy (ISRES) is one such evolutionary optimization algorithm, developed by Runarsson and Yao, 2005. It uses stochastic ranking and island hopping to solve nonlinearly constrained optimization problems. It is an approach to fit experimental data to a rule-based model and obtain the best-fit parameter set. We modified this algorithm to make it faster and more accurate. We tested this modified algorithm—ISRES-plus—to verify the improved performance using two models from literature for this: Schmierer et. al, 2008 model for TGF- signaling in HaCat Cells and Manu, et. al, 2009 model for gap gene segmentation in Drosophila Melanogaster. We generated a best-fit parameter set with ISRES and ISRES-plus and verified that ISRES-plus is faster, robust, and more accurate than ISRES.

503A Genome-wide Effects of the GeneSwitch GAL4 System on *Drosophila melanogaster* Gene Expression *Caroline Pitton*, Sara Gregory, Zachary Drum, Joseph Coolon Wesleyan University, Middletown, CT

Our understanding of gene function in model organisms has increased dramatically over the last 30 years utilizing new reverse genetic techniques to manipulate gene expression. Selectively changing the expression of a gene in an

otherwise wild-type organism allows for much greater understanding of that gene's function and downstream effects on phenotypes than mutant analysis alone. This is especially important for studying essential genes, pleiotropic genes and genes with tissue, stage, sex or enviroment specific effects. In the Drosophila melanogaster model system, use of the GAL4-UAS system is one such genetic tool for experimental manipulation of gene expression that is used in a large number of studies to date. A recent improvement added a hormone-induced (mifepristone) GAL4 expression system called GeneSwitch making it even more tractable and applicable to many experimental questions. Because the GAL4-UAS system was taken from yeast, mifepristone from mammals and both used in D. melanogaster, it is largely considered to act specifically with few if any off-target effects. To test the effects of GAL4 protein expression and mifepristone exposure on genome-wide gene expression, we performed RNA sequencing (RNA-seq) and identified numerous D. melanogaster genes that are significantly differentially expressed in response to GAL4 and RU486. Our study provides evidence that use of the GeneSwitch GAL4-UAS system requires effective control experiments and provides suggestions for future studies to maximize the utility of this genetic toolkit.

504B A homeostatic transcriptional response counteracts I-SMAD activity in Drosophila motor neurons *Jacqueline Kanzler*, Zainab Seyal, Mikolaj Sulkowski Southern Connecticut State University

Given its pleiotropic nature, TGF-b signaling must be precisely regulated both intra- and extracellularly to have the proper effect on target cells. Inhibitory SMADs (I-SMADs) are a potent intracellular regulator of TGF-b signaling. However, there are numerous hypotheses regarding the mechanism by which I-SMADs function. Although biochemical studies show that Dad, the sole *Drosophila* I-SMAD, inhibits the receptors that phosphorylate the effector molecule Mad, our in vivo results indicate that Dad functions downstream of the receptors. We have found a paradoxical increase in phosphorylated Mad (pMad) in motor nuclei following Dad overexpression. qPCR analysis supports the hypothesis that a homeostatic transcriptional response counteracts I-SMAD activity in motor neurons.

505C Characterization of a *Drosophila* Activin signaling network *Yisi Louise Lu*, Hiroshi Nakato, Michael O'Connor University of Minnesota, Minneapolis, MN

Drosophila is an ideal model system for the study of TGF-β signaling networks and their roles in organism development and homeostasis. In *Drosophila,* there are three TGF-β/Activin-like ligands, Myoglianin (Myo), activin-β (Actβ), and Dawdle (Daw). These factors have been implicated in 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-like ligands produce specific responses and phenotypic outcomes in different tissues. We hypothesize that tissue-specific responses result from several attributes of the signaling network. First, there are cross regulatory interactions between the different ligands. Second, each ligand preferentially signals through one of three isoforms of the Type I receptor encoded by the Babo locus. Third, each tissue expresses its own complement of Babo isoforms. Finally, the transcriptional transducer dSmad2 associates with different tissue-specific cofactors to stimulate an appropriate downstream response for that tissue. The lab's long-term goal is to elucidate how these three ligands coordinate to produce the appropriate balance of signals to maintain viability and physiological homeostasis.
We have begun to investigate the cross-regulation relationships among the three Activin ligands by qPCR studies as well as by employing transgenic GFP reporter fly lines. Our preliminary data suggested 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. We have also begun efforts to identify direct downstream targets of Activin signaling through transcriptomic and ChIP-seq analyses. Preliminary analysis of this data will be presented. These studies will provide a deeper understanding of the Activin signaling network and the regulatory interactions that it provides in controlling important physiological activities such as proliferation and energy utilization in several key tissues including muscle, fat and brain.

506A Determining how antagonistic transcription factors control transcription dynamics for robust cell fate specification by single nuclei imaging of transcription factor and target mRNA dynamics *Suzy SJ Hur*[1], Sebastian Bernasek [2], Nicolás Peláez[3], Richard W. Carthew[4], Ilaria Rebay[1] 1) Ben May Department for Cancer Research, University of Chicago, Chicago, IL; 2) Department of Chemical and Biological Engineering, Northwestern University, Evanston, IL; 3) California Institute of Technology, Pasadena, CA 91125, USA. Hanna H. Gray Fellow of the Howard Hughes Medical Institute (HHMI); 4) Department of Molecular Biosciences, Northwestern University, Evanston, IL

During animal development, a highly organized pattern of tissue-specialized cell fates emerges from a population of initially equivalent progenitor cells. This is mediated by transcription factors (TFs) acting downstream of spatially localized extracellular signals to produce spatially refined expression of fate determining genes. Each gene is transcribed in discontinuous bursts with gene-specific temporal dynamics. How TFs control the mRNA expression dynamics of their

targets, and how these dynamics in turn contribute to the specificity and robustness of cell fate transitions remains an important question in developmental biology.
The Drosophila eye development is an ideal system to address this question. In the developing eye disc, a precise complement of eight photoreceptor neuron fates (R1-R8) are specified in each ommatidium from a population of multipotent progenitor cells through the reiterative use of RTK/MAPK signaling. Transition from a progenitor to a specified state is mediated by an antagonistic pair of TFs, Pointed and Yan, which compete for the same binding site and exert opposing functions downstream of RTK/MAPK signaling. Yan represses gene expression to maintain cells in a progenitor state while Pointed activates the expression of distinct photoreceptor fate genes to trigger the transition to a specified state.
We found that increased Pointed-to-Yan ratio in the nucleus, rather than the absolute concentration of either TF, promotes the transition to a specified state. Focusing on the R3/R4 outer and on R7 inner photoreceptor fates, we show that genetic perturbations that elevate the ratio prematurely in the progenitors induce ectopic fate transitions while perturbations that prevent the ratio increase bias cells to remain in the progenitor state. By combining immunostaining, single-molecule RNA fluorescent in-situ hybridization (smFISH), and quantitative imaging, we will simultaneously measure the protein levels of Pnt and Yan and nascent mRNA levels and bursting frequency of the R3/R4 gene *salm* and the R7 gene *pros* in individual cells that remain in the progenitor state vs. those entering the specified R3/R4 and R7 states. By comparing tissues with wild type and altered Pnt and Yan levels, we will present our latest mechanistic models for how distinct mRNA transcription dynamics downstream of Pnt and Yan direct precise and reliable tissue patterning.

507B In vitro identification of critical cis elements in the embryonic Drosophila histone locus *Pamela Diaz-Saldana*[1], Tsutomo Aoki[2], Paul Schedl[2], Leila E. Rieder[1] 1) Dept. of Biology, Emory University, Atlanta GA; 2) Dept. of Molecular Biology, Princeton University, Princeton, NJ

The timing of the nuclear cycles during early Drosophila embryonic development relies heavily upon the availability of histones. The five canonical histone genes are clustered into an array, and ~100 arrays are arranged in tandem at the single histone locus on chromosome 2L. Prior to zygotic genome activation, the histone locus is activated, which includes recruiting a suite of transcription and processing factors collectively known as the histone locus body (HLB). Even though embryonic HLB formation is an evolutionarily conserved event, we do not yet understand the molecular interactions that mark the histone locus for early and unique regulation. Previous in vivo work using transgenes pinpointed specific cis elements (GA-repeats) in the histone3/histone4 promoter that identify the locus for HLB formation by recruiting the transcription factor CLAMP. CLAMP also participates in male dosage compensation. In order to probe the interaction between histone locus and transcription factors more precisely, we tested histone array sequences in vitro for their ability to bind proteins in embryo extract. We performed gel shift assays using histone array probes, confirming the requirement for the GA-repeat cis elements in the histone3/histone4 promoter. In addition, we performed supershifts and observed that both CLAMP and the GA-repeat binding protein GAGA factor bind to this region in vitro. Interestingly, both of these proteins are members of the late boundary complex (LBC), a giant insulator complex that forms in late embryos and participates in both fab7 boundary function and dosage compensation. In the future, we will test the ability of recombinant CLAMP alone to target cis elements in the histone3/histone4 promoter, which it may do prior to LBC formation.

508C Investigating the Effects of Genetic Distance and Regulatory Elements on Tandem Gene Duplicate Expression in *Drosophila Melanogaster* *Georgia McClain*, David Loehlin Williams College

The ways in which a gene's position in the genome and the surrounding genomic contents can affect its expression is not well understood. Tandem duplicate genes are a good model for studying these phenomena, as previous studies have demonstrated evidence of a nonlinear relationship between gene copy number and protein expression. Furthermore, there are several known examples of human diseases associated with copy number variation and relative gene overexpression, so a better understanding of gene duplication and its effects may provide valuable information in addressing the specific causes of these disorders. The Loehlin lab is attempting to investigate the characteristics of tandem *Adh* gene duplicates in *Drosophila melanogaster* that contribute to deviation from two-fold expression.
We are investigating two qualities of synthetic tandem *Adh* duplicates: distance between duplicates, and regulatory element duplication. To do this, we inserted FRT constructs on either side of the *Adh* gene of *Drosophila melanogaster* and used them to create recombinant flies. To test whether the distance between tandem *Adh* copies affects relative protein expression level, we designed recombinants with *Adh* genes at different distances from one another. To test whether the duplication of *Adh* regulatory elements affects expression, we keep the distance between the duplicates the same, but vary whether or not the regulatory region was duplicated. We can then measure the ADH enzyme levels in the flies in order to determine whether ADH expression is influenced by distance between the gene duplicates and/or duplication of regulatory elements.
Preliminary results show that our method successfully creates recombinant *Drosophila melanogaster* individuals that have tandem duplicate *Adh* genes, and that these duplicates have higher expression levels relative to individuals with a single copy.

509A P-bodies Protect mRNAs from the RNAi Machinery Livia Bayer, *Samantha Milano*, Diana Bratu Hunter College, CUNY

In the last two decades many studies have utilized the powerful method of knocking down gene expression via RNAi. However, the level of knockdown varied between genes leading to a range of mRNA levels from full knockdown to very minimal decrease in the target mRNA. We found that two post-transcriptionally regulated mRNAs are protected in P-bodies, giving us a possible hint as to why certain mRNAs are not susceptible to RNAi. This hypothesis stemmed from our initial finding that *oskar* mRNA was only degraded after it localized to the posterior pole. *oskar* mRNA is transcribed and transported to the oocyte throughout oogenesis and it is localized to P-bodies. Since ribosome entry into P-bodies is inhibited, it is reasonable for us to hypothesize that at the posterior pole, where *oskar* mRNA is translated, the transcript has to be released from P-bodies, thus allowing access to the transcript for the RNAi machinery. Our finding that mRNAs localized in P-bodies are not degraded by RNAi came as a surprise to us. Numerous proteins that are components of the RNAi machinery localize into P-bodies, and we expected a strong knockdown when we initiated *oskar* RNAi. During early investigations into P-bodies, it was hypothesized that P-bodies are sites of mRNA decay due to the presence of numerous decay enzymes such as Dcp 1, Dcp 2 and Xrn 1. Recently, experiments carried out in live cells did not find mRNA degradation in P-bodies. Furthermore, it would be also of interest to assess if other translationally repressed mRNAs (i.e. *nanos*) are also protected from RNAi in P-bodies. Our results indicate that P-body protection is maintained in the nurse cells, but investigation of mRNA levels in the nurse cells could reveal further insights into this intriguing mechanism.

510B The mRNA regulatory function of Brat is essential for development and neurogenesis *Robert Connacher*[1], Yichao Hu[2,3], Richard Roden[1], Xiaohang Yang[3], Howard Lipshitz[2], Michael O'Connor[1], Aaron Goldstrohm[1] 1) University of Minnesota, Minneapolis, MN; 2) University of Toronto, Toronto, ON, Canada; 3) Zhejiang University, Hangzhou, China

TRIM-NHL proteins share a conserved domain architecture and play crucial roles in stem cell biology, fertility, and development. Recently, multiple TRIM-NHL proteins were shown to recognize specific RNA motifs and structures via their NHL domain. TRIM-NHLs negatively regulate their bound mRNAs. In light of this observation, we sought to determine the biological role of their RNA-binding activity. Additionally, we investigated the mechanism by which TRIM-NHLs repress mRNAs.

We focused on the *Drosophila* TRIM-NHL protein Brain Tumor (BRAT), which controls development and stem cell fate. First, RNA-binding defective mutations were introduced into the endogenous *brat* locus via CRISPR/Cas9 genome engineering. Our phenotypic analysis demonstrated that the key residues necessary for RNA-binding *in vitro* are essential for survival and larval neurogenesis. RNA-binding defective mutations phenocopy the lethality observed in loss-of-function *brat* mutations. Additionally, these mutations produce ectopic neuroblasts and brain tumors similar to classical *brat* mutants. These results demonstrate the essential function of BRAT derives from its ability to bind and regulate mRNAs.

We further used cell-based reporter assays to elucidate the molecular mechanism by which BRAT controls protein expression. We find that BRAT-mediated repression depends on the 3' poly-adenosine tail of target mRNAs and components of the CCR4-NOT deadenylase complex. We also identified the domains of BRAT that are necessary to repress target mRNAs. Together, our data support a mechanism wherein BRAT recruits deadenylases to reduce translation and promote decay of target mRNAs.

Collectively, our findings provide crucial insights into the molecular mechanism and function of BRAT *in vivo*.

511C Protein-RNA interaction drives co-transcriptional regulation and RNA processing *Annie Huang*, Mukulika Ray, Erica Larschan, Nicolas Fawzi, Noah Wake, Victoria Johnson Molecular Biology, Cell Biology & Biochemistry Department, Brown University, Providence, RI

Biomolecular condensates are membrane-less compartments formed by the biophysical phenomena of phase transition mainly through Protein-RNA interactions. Both transcriptional complexes and RNA processing units are reported to form such condensates. Several transcription factors and RNA binding proteins that contain IDR (**I**ntrinsically **D**isordered **R**egions) have been reported to have a role in phase transition, and thus it is interesting to study how RNA-protein complexes can potentially form and dissolve such condensates. In the present study, we have explored the potential of DNA binding transcription factor protein CLAMP (**C**hromatin **L**inked **A**daptor for **M**SL **P**roteins), which contains IDR, in *Drosophila Melanogaster* as a protein that can form bio-condensates. CLAMP recruits the MSL (**M**ale **S**pecific **L**ethal) Complex, an RNA-protein complex consisting of long non-coding RNAs roX1 and roX2 along with five other proteins, to the male X-chromosome resulting in the transcriptional upregulation of the male X-chromosome in a process known as dosage compensation. Therefore in this project, we studied the interaction between CLAMP and roX2 RNA to test the hypothesis that these two components might drive the process of dosage compensation via phase separation. Also, CLAMP regulates sex-specific splicing, a process regulated by ribonucleoprotein complexes called

spliceosomes that coordinate co-transcriptional RNA processing via the mechanisim of alternative splicing. In flies and mammals many RNA-binding proteins are part of the alternative splicing spliceosome complex along with non-coding RNA components. In *Drosophila*, CLAMP binds to several of such RNA binding proteins that are reported to interact with a long non-coding RNA hsrω-n. Thus, we also explored CLAMP's binding with hsrω-n to test CLAMP's potential as a legitimate player in phase transition and formation of biomolecular splicing condensates. Using electron mobility shift assays (EMSA) we found that CLAMP and the CLAMP domain that contains the IDR physically bind to both roX2 and hsrω-n non-coding RNAs, reinforcing our hypothesis that CLAMP is a bifunctional protein with DNA/RNA binding capacity and has the potential of regulating transcription and co-transcriptional RNA processing by manipulating bio-physical properties of RNA-protein complexes.

512A Nonsense-mediated mRNA decay plays an essential role during female germline development in *Drosophila melanogaster* *Omar Omar*[1,2], Arwa Abdelhamid[2], Emily Makowicz[2], Diana Bratu[1,2] 1) The Graduate Center, CUNY; 2) Hunter College, CUNY

Across eukaryotes, nonsense-mediated mRNA decay (NMD) is a vital cellular surveillance process. Its initial role was presumed to be mitigation of truncated proteins by degrading aberrant transcripts encoding for them. On an organismal level, NMD is necessary for proper growth and development but how this is accomplished remains poorly understood. Our studies in flies have found that efficient knockdown of various NMD factors results in embryonic lethality as well as perturbations of essential maternally-derived factors, including localized mRNAs necessary for body axis patterning. Moreover, these knockdowns result in improper germline cyst formation due to problems during early mitotic events initiated in the female germline stem cell niche. Taken together, our data suggest that Upf1 protein functions in regulating mRNA stability of transcripts necessary for both proper maternal embryonic axis patterning and germline development.

513B Bruno 1 and Cup interdependent regulation of oskar mRNA life cycle *Livia Bayer*[1], Irina Catrina[2], Stephen Formel[3], Rishi Ravichandran[4], Juan Cambeiro[1], Lizaveta Slinko[1], Diana Bratu[1] 1) Hunter College; 2) Yeshiva University; 3) Tulane University; 4) Sloan Kettering Institute

In D. melanogaster, a subset of mRNAs essential for embryo development are spatio-temporally controlled during oogenesis. oskar mRNA is a critical transcript for germplasm assembly, which is translationally regulated by Bruno 1 and Cup. The current model suggests that Bruno 1 is the key factor for recruiting Cup to oskar mRNA that enables the formation of the translational silencing complex. Here, we address the spatio-temporal requirements for the formation of the Bruno 1-Cup complex contributing to a more universal understanding of mRNA regulation. Our work reveals that the Bruno 1-Cup interaction, as well as their interdependent influence on each other's protein expression, lead to a precise oskar mRNA regulation. Cup mediates recruitment of oskar mRNA to P-bodies driving the translational repression and stability of oskar mRNA and inhibits Bruno 1's role in the activation of translation. When Cup is removed from the complex, Bruno 1 is free to interact with Orb, thus facilitating translational activation.

515A Exploring the novel role of a putative tRNA methyltransferase in synaptic growth and neuronal development *Jennifer Dumouchel*[1], Kimberly Rose Madhwani[2], Caley Hogan[3], Jenna Lentini[4], Kevin Welle[5], Dragony Fu[4], Kate O'Connor-Giles[6,7] 1) Therapeutic Sciences Graduate Program, Brown University, Providence, RI; 2) Neuroscience Graduate Program, Brown University, Providence, RI; 3) Laboratory of Genetics, University of Wisconsin-Madison, Madison, WI; 4) Department of Biology, Center for RNA Biology, University of Rochester, Rochester, NY; 5) Mass Spectrometry Resource Laboratory, University of Rochester Medical Center, Rochester, NY; 6) Department of Neuroscience, Brown University, Providence, RI; 7) Carney Institute for Brain Science, Providence, RI

Dynamic regulation of neuronal genes during transcription and translation is required for proper nervous system development and function. Investigation of transfer RNA (tRNA) modifying enzymes has highlighted the importance of chemical modifications for tRNA stability and protein synthesis. Yeast tRNA methyltransferase 9 (Trm9) methylates wobble uridines in anticodon loops to reinforce cognate codon-anticodon pairings. In metazoa, two Trm9 paralogs, ALKBH8 and TRMT9B have been identified. We identified TRMT9B in an RNAi screen as a negative regulator of synaptic growth, and find that it also plays a key role in regulating neurotransmission. While TRMT9B has been studied for its role as a tumor suppressor, its biochemical role has remained unknown. To study the role of the expanded family, we have generated mutants in both paralogs and assessed changes in bulk tRNA chemical modifications by mass spectrometry. Wobble uridine methylation is absent in *ALKBH8* mutants, consistent with studies in mammals. In contrast, we do not observe deficits in wobble uridine methylation in *TRMT9B* mutants under basal conditions. Thus, TRMT9B may catalyze wobble uridine modification of a small subset of tRNA isodecoders not detectable by bulk nucleotide analysis or evolved a new function. Wild-type, but not methyltransferase-dead, transgenes rescue synaptic overgrowth in *TRMT9B* mutants, indicating that TRMT9B functions through a methyltransferase-dependent mechanism. Unbiased homology modeling of TRMT9B's methyltransferase domain revealed a strong conservation of secondary structure with the reported yeast Trm9 X-ray crystallography structure; consistent with a role as a methyltransferase. To assess TRMT9B function, we turned to transcriptomic and proteomic studies, and will present our latest findings. We investigated transcriptome and proteome

changes in *TRMT9B* null adult fly heads by RNA sequencing and high resolution mass spectrometry, respectively. Differentially expressed genes and proteins are consistent with TRMT9B's role at synapses and prior studies of yeast Trm9, which modulates translation of specific mRNAs in response to DNA damage and oxidative stress. Together, our studies reveal a novel role for tRNA methyltransferase family member TRMT9B in the regulation of synaptic growth and neuronal function.

516V A toolkit to wire synthetic transcriptional circuits in Drosophila melanogaster *Aya Gomaa*[1,2,3], Ariane Ramaekers[3], Radoslaw Ejsmont[2] 1) University of Paris, Paris.; 2) Le Centre de recherches interdisciplinaires, Paris.; 3) Curie Institute, Paris.

Development is an extraordinarily complex and tightly regulated process. A significant part of developmental regulation depends on gene regulatory networks (GRNs), consisting of interacting transcription factors governing spatial and temporal gene expression. Synthetic biology opens a new way to study GRNs: on top of deciphering natural GRNs, scientists reconstruct synthetic ones using engineered transcription factors and target genes. Existing synthetic GRNs have primarily been established in prokaryotes that benefit from toolkits consisting of 'easy to assemble' well-studied genetic elements, such as promoters and transcription factors.
In our project, we are developing a toolkit to reconstruct synthetic GRNs in vivo, in Drosophila melanogaster (D.mel). Our toolkit enables massive combinatorial assembly of synthetic transcription factors (sTFs) using a library of DNA binding domains, activating or repressing domains and a collection of engineered cis-regulatory binding sites that are absent from the (D.mel) genome. We are establishing the gene expression profiles derived from these sTFs individually or in a variety of combinations. Ultimately, sTFs will be used to recreate motifs that occur naturally in GRNs and logic gates. By providing this sTFs library we hope to facilitate the implementation of synthetic GRNs in D.mel and other model organisms, thus enabling in vivo modeling of the native developmental networks.

517V Determinants of transcription factor function *Lauren Hodkinson*, Leila Rieder Emory University

Despite the crowded nuclear environment, transcription factors locate and bind cis elements across the genome while performing context-specific functions. 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 GA-rich cis elements while performing several distinct functions throughout the genome. CLAMP primes the male X-chromosome for dosage compensation, regulates the accessibility of promoters genome-wide, and promotes formation of the conserved histone locus body (HLB), which regulates expression of the replication-dependent histone genes. Although CLAMP targets similar cis elements in all three contexts, it recruits very different locus-specific transcription factors. 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 locus 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 proteins found in the other CLAMP binding contexts on the X chromosome. When we replaced the H3H4p with an X-linked CLAMP recruiting region, HLB factors were not recruited but X chromosome factors were 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 factors 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 trasction using qRT-PCR to further evaluate locus function.

518V *De novo* discovery of motifs enriched in promoters of *D. ananassae* F Element genes *Annabelle Laughlin*[1], Wilson Leung[1], Chris Shaffer[1], Cindy Arrigo[2], Genomics Education Partnership 1) Washington University in St. Louis, St. Louis, MO; 2) New Jersey City University, Jersey City, NJ

The *Drosophila melanogaster* Muller F Element exhibits mostly heterochromatic characteristics (e.g., high repeat density, low recombination rates), but the distal ~1.3 Mb region contains ~80 protein-coding genes that show expression levels similar to that of euchromatic genes. Interestingly, this region has expanded to ~19.1 Mb in *Drosophila ananassae* due to an increase in repeat density (particularly retrotransposons). This project seeks to understand the regulatory mechanisms that allow the successful transcription of F Element genes in such repeat-rich domains by conducting comparative analysis of *D. ananassae*, *D. bipectinata*, *D. takahashii*, *and D. kikkawai*, (where the F Element has expanded to different degrees), focusing on transcription start sites (TSS). The GEP annotation protocol uses experimental data (RAMPAGE, ATAC-Seq, RNA-Seq) and sequence similarity to other *Drosophila* species to define TSS positions and promoter-flanking regions. Over the past two summers, 97 unique TSSs in *D. ananassae* have been annotated. The TSS data were used in coding region annotations. For example, the locations of the putative TSSs relative to the available start codons was used as evidence to support the hypothesis that the G isoform of the *D. melanogaster Zyx* gene

does not exist in *D. ananassae*. In a preliminary analysis using 26 unique TSSs in the *D. ananassae* F Element scaffold QMES02000012, ~77% of the TSS positions and ~86% of the narrow promoter-flanking regions were defined based on RAMPAGE or ATAC-Seq data. In contrast, ~54% of the wide promoter-flanking regions were defined by manual analyses (e.g., BLAST searches, multiple sequence alignments). A *de novo* motif discovery analysis of the 50 RAMPAGE peaks in QMES02000012 identified three significant motifs. Analysis of these motifs using TomTom showed that the two most significant motifs have no similarity to known *D. melanogaster* motifs, while the third showed similarity to a putative Top2 motif abundant at heterochromatic TSSs in *D. melanogaster*. These motifs are being used in searches of the genomes of the other three species to see if they are enriched near the promoters of F Element genes. Comparative analysis of *Drosophila* F Elements could identify regulatory motifs and promoter architecture that are unique to the F Element and that facilitate expression in heterochromatic domains.

519V Regulation of gene expression by the HP1 variants John Schoelz[1,2], *Annesha King*[1,2], Nicole Riddle[1,2] 1) University of Alabama-Birmingham; 2) Department of Biology

Heterochromatin Protein 1 (HP1) was discovered in Drosophila and is a major component of heterochromatin. In *Drosophila melanogaster,* there are three somatically expressed HP1 proteins: HP1a, HP1b, and HP1c. HP1a is essential in flies and is necessary for genomic integrity. 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 is found in heterochromatin and euchromatin as well and acts as repressor and activator of gene expression depending on the target. HP1c is found in both chromatin compartments as well, but its function seems to be mostly in gene activation. 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. In this study, we assessed gene expression changes by recruiting HP1 proteins to endogenous genes. These models suggest that HP1b is of particular importance, but that genomic features including promoter motifs, accessibility, and sequence composition also contribute. To address the model predictions, we used dCas9 to recruit HP1 proteins to specific targets in the genome. We found that dCas9 tethered HP1 proteins have different transcriptional outcomes depending on gene target.

520V Regulation of PDF neuropeptide production in the central nervous system *Jae Park*[1], Gyunghee Lee[1], Siuk Yoo[2] 1) University of Tennessee; 2) Yeongnam University

Pigment-dispersing hormone was first characterized in Crustaceans for its role in the daily rhythms of the retinal pigment dispersion. Insect gene encoding PDH-homolog, PDF, was first characterized in *Drosophila*. PDF is an important output factor for the maintenance of the circadian rhythms via synchronizing various clock-gene expressing neurons. Expression of PDF was found in the three distinct groups of neurons in the central nervous system; s-LNvs and l-LNvs in the brain lobe, and abdominal ganglionic neuron (PDFab). However, regulation of PDF production is not well understood. We have identified a cis-acting regulatory element (PRE) that is critical for the PDF transcription. We also identified NK2.1 homeodomain (HD) transcription factor, *scarecrow* (*scro*), as a binding factor to the PRE. Transgenic expression of wild-type *scro* eliminated PDF expression, while that of homeodomain-deficient scro failed to do so. A construct containing HD-alone also suppressed PDF expression, supporting that scro suppresses PDF expression via the interaction of HD with PRE in PDF-negative neurons. While loss-of-function mutations caused abnormal development of the optic lobes, ectopic expression of scro in different gal4 domains caused various developmental defects, such as rough eye phenotypes and extension of ventral nerve cord. We also observed that knockdown of *disco-related* (*disco-r*) gene suppressed PDF expression, raising the possibility of *disco-r* as a putative PDF transcription activator. To understand the maturational mechanisms of PDF precursor, we generated an antibody that is intended to detect only PDF-associated peptide. The antibody detected all endogenous cell bodies and axonal projections, suggesting that PDF maturation takes place in the vesicles while transporting to the axon terminals. This reagent will be used to study the genetic and molecular mechanisms of the maturational processes of PDF precursor in the clock neurons.

521V It's *abo*ut time: an investigation into the role of *abnormal oocyte* (*abo*) in embryonic histone gene regulation *Eric Albanese*, Casey Schmidt, Leila Rieder Emory University

Abnormal oocyte (*abo*) is a maternal effect gene first characterized by Larry Sandler in 1970. Loss-of-function mutations in *abo* cause defects in embryonic development. Later characterization of *abo* revealed that it negatively regulates histone gene expression—likely through targeting *histone* promoters. Interestingly, the viability defects prompted by *abo* mutation can be rescued by an overexpression of heterochromatic regions, suggesting that heterochromatin can act as a "sponge" to soak up the excess histones. Despite these important experiments, *abo* has received little attention since the early 2000s, and its precise role in 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 probe *abo* localization through staining polytene chromosomes and determine abo expression levels by western blot. We also utilize available *abo* mutants and RNAi lines to determine the molecular mechanism of histone gene repression. Overall, our work will contribute to a growing body of knowledge regarding histone gene regulation in early embryo

development.

522V Differential regulation of alternative promoters emerges from unified kinetics of enhancer-promoter interaction Jingyao Wang[1,2], Shihe Zhang[2,3], Hongfang Lu[1,2], *Heng Xu*[2,3] 1) School of Life Sciences and Biotechnology, Shanghai Jiao Tong University, Shanghai, China; 2) Institute of Natural Sciences, Shanghai Jiao Tong University, Shanghai, China; 3) School of Physics and Astronomy, Shanghai Jiao Tong University, Shanghai, China

Many eukaryotic genes contain alternative promoters with distinct expression patterns. How these promoters are differentially regulated remains elusive. Here, we apply single-molecule imaging to quantify the transcriptional regulation of two alternative promoters (P1 and P2) of the Bicoid (Bcd) target gene *hunchback* in syncytial blastoderm *Drosophila* embryos. Contrary to the previous notion that Bcd only activates P2, we find that Bcd activates both promoters via the same two enhancers. P1 activation is less frequent and requires binding of more Bcd molecules than P2 activation. Using a theoretical model to relate promoter activity to enhancer states, we show that the two promoters follow common transcription kinetics driven by sequential Bcd binding at the two enhancers. Bcd binding at either enhancer primarily activates P2, while P1 activation relies more on Bcd binding at both enhancers. These results provide a quantitative framework for understanding the dynamics of complex eukaryotic gene regulation.

523V Reporter gene assays and chromatin-level assays define substantially non-overlapping sets of sequences as enhancers *Daniel Lindhorst*[1], Marc S. Halfon[1,2] 1) University at Buffalo-State University of New York; 2) NY State Center of Excellence in Bioinformatics & Life Sciences

Enhancers are critical for eukaryotic transcriptional regulation. However, just how enhancers should be defined remains unclear. While reporter gene assays, which are function based, have been the traditional benchmark for enhancer identification, next-gen sequencing-based techniques that scan for open chromatin, histone modifications, or specific transcription factors (e.g. ATAC-Seq, ChIP-Seq) have become a new source of powerful, high-throughput methods for defining enhancers. Whether these various enhancer definitions consistently lead to identification of the same sequences is unknown. To compare the functional and the chromatin-level enhancer definitions, we analyzed the overlap between enhancers defined in two enhancer databases, REDfly (Rivera et al. 2019, *NAR* 47:D828) and EnhancerAtlas2.0 (Gao and Quan 2019, *NAR* 48:D58). REDfly uses primarily a functional definition based on reporter gene analysis, while EnhancerAtlas integrates the results of chromatin-level assays using a supervised learning model. We used REDfly's search capabilities to build tissue-specific enhancer datasets and compared these with tissue-specific EnhancerAtlas datasets. Surprisingly, we found that only 4 of 11 sets (36%) showed statistically significant overlap. From this, we hypothesized that the observed discrepancies could be caused by the way data from multiple techniques/assays are integrated by the EnhancerAtlas method. We took the underlying EnhancerAtlas data subsets and compared them individually with their matched REDfly sets. 66% of the EnhancerAtlas subsets had significant overlap, a substantial increase from the previous, full-set comparisons, although still limited. However, these EnhancerAtlas subsets only had a median intersection with REDfly enhancers of 39%. Thus, even the sets with significant overlap include fewer than half of the expected reporter-gene defined enhancers from the corresponding REDfly set. We derive two conclusions from our findings. First, during the integration of the EnhancerAtlas data sets, enhancers present in the underlying EnhancerAtlas data are being lost, suggesting that a more sensitive learning model may be required. More importantly, the poor overlap between the reporter-gene defined enhancers and the chromatin-assay defined enhancers suggests that one or both of these approaches carries a high error rate. Further investigation will be required to determine which approaches lead to the most accurate enhancer definition.

524V Temporal-specific requirement of Bruno1 in *Drosophila* flight muscle to support myofibril assembly, growth and maturationTemporal-specific requirement of Bruno1 in *Drosophila* flight muscle to support myofibril assembly, growth and maturation Elena Nikonova[1], Marc Canela Grimau[2], Tobias Straub[1], *Maria Spletter*[1] 1) Ludwig-Maximilians-University Munich; 2) University of Barcelona

Animals have different types of muscle fibers with distinct morphologies and contractile properties. These characteristics are established during development through regulation of alternative splicing and gene expression. Patterns of mRNA isoform expression differ between myofiber types, change as muscles differentiate, mature and age, and are often disrupted in muscle disease, suggesting that RNA regulation and in particular alternative splicing helps define muscle functional properties. It is therefore important to examine how RNA processing functions in healthy muscle, to better understand how misregulation contributes to muscle disease. Misregulation of the RNA-binding protein CELF1 is thought to underly myotonic dystrophy (DM1), but its function in muscle development is incompletely understood. Using *Drosophila melanogaster* as a tractable genetic model, we have previously shown using RNAi knockdown that the CELF1 homologue Bruno-1 (Bru1) controls flight muscle specific alternative splicing, regulating both sarcomere growth and myosin contractility. We generated new CRISPR-mediated alleles in Bru1 that affect all Bru1 isoforms, resulting in stronger phenotypes in mutant flies that revealed structural defects at the earliest stages of myofibril formation, notably disorganization of the actin cytoskeleton that adversely affects myofibrillogenesis in differentiating IFM. Using temporally-restricted RNAi knockdown and rescue experiments, we demonstrate that there are distinct requirements

for Bru1 mediated splicing during early and late stages of myofibril formation. After sarcomere formation, aberrant actin incorporation arrests growth in thin-filament length, but promotes lateral growth leading to formation of hollow myofibrils. We performed mRNA-Seq and mass spectrometry and identified misregulation of both sarcomeric proteins and other RNA-processing factors. Moreover, our temporal transcriptomics data reveal a progressive misregulation of gene expression and splicing as IFM development proceeds. Taken together, our data indicate that during muscle differentiation, Bru1 regulates cytoskeletal rearrangement necessary for myofibril formation as well as the balance in length versus lateral growth of the thin-filament. Defective RNA processing thus causes sarcomeric structural defects and progressive malfunction of dystrophic muscle.

525V RNA-binding protein Nocte regulates *glass* mRNA translation during *Drosophila* eye development *Tianyi Zhang*, Shuaikun Su, Katherine Ho, Seung-Kyu Lee, Weiping Shen, Weidong Wang National Institute of Aging

Drosophila nocte encodes a 250 kDa protein with an N-terminal BAT2 domain, RGG motifs in the middle, and large low-complexity regions. Studies in *Drosophila* showed that a partial mutation of *nocte* leads to defects in temperature compensation of the circadian clock. A recent study shows that mouse Nocte orthologs PRRC2A is an m6A reader and its binding can stabilize specific mRNAs; and its inactivation disrupts neural development. However, the molecular functions of Nocte and its orthologs are still largely unknown. Our results from IP-Mass Spec experiments of RNA-binding proteins Top3b and TDRD3 identify Nocte as their strong binding partner. IP-Mass Spec experiment of Nocte shows that it interacts with other RNA-binding proteins and ribosomal proteins, supporting that Nocte is an RNA binding protein. We generated *nocte* knockouts by CRISPR and found that *nocte-KO* flies die at larval-pupal stages. Specific knockdown of *nocte* by RNAi in fly eyes induces rough and small eye phenotypes. The RNA-seq results from *WT* and *nocte-RNAi* eye discs indicate that Nocte depletion disrupts *chaoptin* transcription. Immunostaining, qRT-PCR and RIP-qPCR results show that Nocte is necessary and sufficient to promote the translation of *glass* mRNA, which encodes a transcription factor regulating *chaoptin* directly. With reporter assays and CRISPR edited alleles, we demonstrated that Nocte counteracts the suppression effects of the upstream ORF (uORF) at *glass* 5'UTR to maintain *glass* translation. We are investigating the detailed mechanisms of how Nocte regulates *glass* translation and its global effects on mRNA translation by Ribo-seq.

526V Dynamic time warping on sn- and sc-RNA-seq trajectories of Drosophila adult and larvae testis enables contrasting the different germline developmental stages *Soumitra Pal*[1], Amelie Raz[3], Sharvani Mahadevraju[2] 1) National Center for Biotechnology Information, National Library of Medicine, National Institute of Health, Bethesda, MD, 20894, USA; 2) Laboratory of Cellular and Developmental Biology, National Institute of Diabetes and Kidney and Digestive Diseases, National Institutes of Health, Bethesda, MD, 20892, USA; 3) Whitehead Institute for Biomedical Research, Cambridge, MA, USA

Capturing the single nuclear and single cellular transcriptomics using snRNA-seq and scRNA-seq have advantages of their own. In snRNA-seq the message made by a cell at a certain time is captured whereas in scRNA-seq, the message stored for a longer period is captured as well. Similarly, RNA-seq profiles (sn or sc) captured from different stages of the life-cycle can reveal differences in the transcriptome across life stages. However, co-analyzing such diverse datasets together to gain biological insights poses significant challenges as the datasets could have batch effects eclipsing the biological differences, or even have significantly different RNA-features. Here we use an alternate approach where we first analyze the datasets separately utilizing then individual nuances and then contrast the analyses by a post-analysis alignment procedure. Specifically, we align the trajectory of germ cells from different data sets and set out to identify if there are any differences in the germ cell developmental stages revealed by the nuclear and whole cell transcriptomics at either life-stages.

We analyzed three datasets from different labs: 1) snRNA-seq on adult testis from Fly Single Cell Atlas, 2) scRNA-seq on adult testis and 3) scRNA-seq on larval testis. The snRNA-seq germline has 21,061 nuclei whereas the adult and lthe arval scRNA-seq have 6,438 and 10,652 cells respectively. The trajectory analyses using monocle3 on these datasets separately arrange the germ cell that progresses from spermatogonia to spermatids via different developmental stages. However, to contrast these individual trajectories for uncovering differences in biology, we adapted Dynamic Time Warping (DTW). We were able to align the three different trajectories on a common pseudotime scale. The alignment of germline adult and larval scRNA-seq pseudotime revealed cell states in adults (elongating spermatids) that are absent in the larvae confirming the legitimate alignment. Buoyed by the accuracy of the alignment by DTW, we next focussed on the difference between snRNA-seq and scRNA-seq of adult germ cells and identified a set of genes for which the cell stops producing the RNAs in the nucleus while differentiating from spermatocyte to spermatid but shows purdurance of transcript in the cytoplasm. While some of these genes are known in literature, some are our novel findings that we set out to validate experimentally.

527V Identification of candidate regulators of transposable element (TE) expression from host gene/TE coexpression *Matthew Lawlor*, Weihuan Cao, Christopher Ellison Rutgers University

The small RNA pathways involved in *Drosophila* transposable element (TE) control are widely studied, but the role of

host transcription factors in regulating TE expression is not well understood. Prior studies show that related TE families show similar expression patterns and tissue specificity. Furthermore, many TEs show expression patterns biased to specific cell types, likely due to regulation by host-encoded trans-acting factors. We reasoned that TE expression should be associated with natural variation in expression of such host-encoded factors across genetic backgrounds in wild populations of *Drosophila*. To test this hypothesis, we quantified host gene and TE expression in a previously published RNA-seq study of gene expression in 200 *Drosophila* Genetic Reference Panel strains. We employed linear models to identify significant associations between TE expression and host genes while accounting for confounding factors such as TE copy number and intronic insertions. We additionally correct for a systematic bias affecting highly expressed genes. Our approach identifies 34 previously reported piRNA pathway genes which show negative associations with three or more TEs. We additionally identify 32 transcription factors and cofactors (including seven unnamed genes) showing exclusively positive associations with three or more TEs in both sexes. Another 31 transcription factors and cofactors (including three unnamed genes) are negatively associated with TE expression. For 11 of these 63 candidates, we were able to confirm their effect on TE expression using publicly available RNA-seq after RNAi knockdown in S2R+ cells. We are in the process of performing in-vivo, tissue-specific RNAi knockdowns of additional candidates. These novel TE regulators will shed light on many aspects of host/TE co-evolution including mechanisms by which TEs co-opt host transcription factors to mobilize in the germline as well as both antagonistic and synergistic interactions between host gene regulatory networks and TE expression.

528V Integration of BMP, JAK/STAT and EGFR signaling during anterior-posterior patterning of the follicular epithlium. *Kelvin Ip*, Kaitlin MacDonald, Scott De Vito, Mariana Fregoso Lomas, Laura Nilson McGill University

During development, interpretation of positional cues by individual cells generates stereotyped patterns of gene expression and cell fate. We study the Drosophila follicular epithelium, where cells integrate input from multiple extracellular signals to choose between discrete anterior and posterior cell fates.
Central to the patterning of this epithelium is the localized activation of the epidermal growth factor receptor (EGFR) by a ligand, Gurken (Grk), secreted by the underlying oocyte. Early in oogenesis, Grk is localized at the posterior and induces expression of the transcription factor Midline (Mid). Later, Grk moves to the dorsal anterior, where it instead induces the transcription factor Mirror (Mirr). The choice between these alternative outcomes depends on the presence of additional localized secreted ligands. Posterior cells experience Grk in the presence of the JAK/STAT pathway ligand Unpaired (Upd), which cooperates with Grk to induce *mid* while also independently repressing *mirr*, At the anterior, the BMP signaling ligand Decapentaplegic (Dpp) cooperates with Grk to induce *mirr*, while independently repressing *mid*. In addition, there is mutual repression between *mid* and *mirr*. We propose that this joint activation and derepression generates a bistable choice between these two EGFR targets, but how a cell interprets and integrates these signals remains unknown.
We address this question by characterizing reporters bearing putative *cis*-regulatory regions (CRRs) from the *mid* and *mirr* loci. We discovered that, for each gene, the response elements for the known regulatory inputs are distributed across two reporters bearing non-overlapping CRRs. Dpp response elements are located in a different *mid* CRR than those for Upd or Mirr, and Upd response elements are located in a different *mirr* CRR than those for Dpp or *Mid*. Reporters with these CRRs differ in expression boundary and dynamics, providing a tool for assessing the contribution of different regulatory inputs to *mid* and *mirr* expression . Further analysis of these CRRs suggests that repression of *mirr* and *mid* by JAK/STAT signaling and BMP signaling, respectively, requires binding of the signal mediators to *cis*-elements. We will be investigating the interplay between the different regulatory inputs to *mid* and *mirr* and their role in patterning by manipulating these *cis*-elements in different sequence contexts.

529V Myc-regulated miRNAs modulate p53 expression in Drosophila. *Gervé María Paula*, Sanchez Juan, Ingaramo Clara, Dekanty Andres IAL (instituto de agrobiotecnologia del Litoral)

Myc, a conserved transcription factor involved in the regulation of growth and metabolism, has been shown to regulate the biogenesis of miRNAs in cultured mammalian cells, however, the exact mechanisms by which Myc affects miRNA function remain unclear. Here we provide evidences that Myc directly regulates the expression of a high number of miRNAs in Drosophila. ChIP-seq analysis revealed that dMyc is highly enriched in the promoter region of 113 (50%) miRNA genes, and dMyc depletion showed reduced expression of most miRNA genes analysed. Along with reduced pri-miRNA expression, we observed decreased levels of pre- and mature miRNAs and increased expression of miRNA activity sensors (miR-GFP) in myc-depleted cells. Conversely, Myc overexpression increased miRNA levels and reduced miR-GFP expression, strongly suggesting that dMyc modulates the expression and processing of miRNAs. We also show that Myc-dependent regulation of miRNA biogenesis plays a critical role in the response to nutrient stress. Dmp53, the single Drosophila ortholog of mammalian p53, is negatively regulated by miR-305 in the fat body in a nutrient-dependent manner, and its activation is required for maintaining metabolic homeostasis and promoting survival under nutrient deprivation. Our results revealed that dMyc directly binds miR-305 locus and promotes its expression, thus maintaining low Dmp53 levels in the fat body of well-fed animals. Under starvation conditions, however, dMyc protein levels and miR-305 expression are reduced which result in increased Dmp53 levels. These findings demonstrate an essential role for Myc in regulating miRNA expression and highlight the importance of Myc-dependent regulation of miRNA biogenesis in

metabolic homeostasis and organismal survival upon nutrient stress.

530V The NXF gene family in *Drosophila*: Evolutionary History and Cell-Type Specific Gene Expression *Martin Calvino*, Jae Hak Son, Christopher Ellison The Human Genetics Institute of New Jersey, Rutgers University

In eukaryotes, mRNAs are transported from the nucleus to the cytoplasm by a family of nuclear RNA export factors (NXF). Members of this protein family are characterized by an amino-terminal region (NTR), an RNA-binding domain (RBD), leucine-rich repeats (LRRs), the NFT2-like domain (NTF2), and a Ubiquitin-associated (UBA) domain. In *Drosophila melanogaster*, the NXF family is composed of four members that have undergone functional diversification. While Nxf1 is involved in nucleocytoplasmic mRNA export, Nxf2 and Nxf3 were co-opted for piRNA-guided transcriptional silencing of transposable elements (TE). The role of Nxf4 remains to be elucidated. While Nxf1 is ubiquitously expressed, Nxf2 and Nxf3 are predominantly expressed in gonads; and Nxf4 displays testis-specific expression only. In this study, we describe the evolutionary diversification of the NXF gene family across more that 100 species of *Drosophila* and study branch-specific rates of evolution and test for evidence of positive selection among members of this family. We additionally analyzed publicly available single-cell RNA-seq data to identify cell-type specific expression profiles for NXF family members. Our results provide important insight in the evolution of the NXF gene family and its role in transposon suppression.

531V Social experience and pheromone receptor activity reprogram behavioral switch gene splicing and neuromodulatory gene expression in sensory neurons Bryson Deanhardt [1, 3], Qichen Duan[2], *Chengcheng Du*[2], Charles Soeder [3], Corbin Jones[3], Pelin Volkan[1, 2] 1) Department of Neurobiology, Duke University, Durham; 2) Department of Biology, Duke University, Durham; 3) Department of Biology, University of Chapel Hill, Chapel Hill

Social experience and pheromone signaling in pheromone sensing olfactory neurons affect pheromone responses and male courtship behaviors in *Drosophila*. We previously showed that social experience and pheromone signaling modulates chromatin around behavioral switch gene *fruitless*, which encodes a transcription factor necessary and sufficient for male courtship behaviors. Fruitless drives social context dependent modulation of courtship behaviors and pheromone responses in sensory neurons, however, the molecular mechanisms underlying this circuit-level neuromodulation remain less clear. To identify the molecular mechanisms driving social experience-dependent neuromodulation, we performed RNA-seq from antennal samples of mutants in pheromone receptors and *fruitless,* as well as grouped or socially isolated wild-type males. We found that loss of pheromone detection differentially altered the usage levels of *fruitless* exons, suggesting changes in splicing patterns. In addition, many Fruitless target neuromodulatory genes, such as neurotransmitter receptors, ion channels, and ion transporters, were differentially regulated by social context and pheromone signaling. Recent studies showed that social experience and juvenile hormone signaling coregulated *fru* chromatin to modify pheromone responses in olfactory neurons. Interestingly, genes involved in juvenile hormone metabolism were also misregulated in different social contexts and mutant backgrounds. Our results suggest that modulation of circuit activity and behaviors in response to social experience and pheromone signaling arise due to large-scale changes in transcriptional programs for neuromodulators downstream of behavioral switch gene function.

532C A tale of two functions: Epigenetic programming and RNA splicing by Tip60 histone acetyltransferase *Akanksha Bhatnagar*[1], Bhanu Chandra Karisetty[1], Keegan Krick[2], Elizabeth Heller[2], Felice Elefant[1] 1) Drexel University, Philadelphia, PA; 2) University of Pennsylvania, Philadelphia, PA

Reduced histone acetylation causes chromatin packaging alterations in neurons with concomitant transcriptional dysregulation that is a key initial step in Alzheimer's disease (AD) etiology. In this regard, we have previously established a central role for Tip60 histone acetyltransferase (HAT) mediated chromatin dynamics in neuronal function, cognition, and AD. However, it remains to be elucidated if Tip60 has alternative cellular functions other than histone acetylation that could contribute towards AD pathogenesis. Altered RNA splicing has recently been highlighted as a widespread hallmark in AD transcriptome that is implicated in the disease. Strikingly, we discovered a novel RNA binding function for Tip60 in the *Drosophila* brain that is conserved in the human hippocampus and impaired in brains from both, AD fly models and AD patients. Our transcriptomic analysis of the RNA molecules specifically bound to Tip60 by Tip60-RNA immunoprecipitation (RIP) revealed an RNA binding function for Tip60 that was highly specific, selective, and reproducible, with Tip60 RNA targets enriched for critical neuronal processes that are implicated in AD. Remarkably, 79% of Tip60's RNA targets overlap with its chromatin gene targets, supporting a model by which Tip60 orchestrates bi-level transcriptional regulation at both the chromatin and RNA level, a function unprecedented for any HAT to date. Since RNA splicing typically occurs co-transcriptionally and defects are a recent hallmark of AD, we hypothesized that Tip60 regulates splicing of its RNA targets and that this process is disrupted in AD. Transcriptome analysis from *Drosophila* brains that model AD showed reduced Tip60 levels and intriguingly, revealed that the residual Tip60 targets a different set of RNA when compared to wild-type flies. Additionally, we observed significant Tip60-associated RNA splicing defects in the *Drosophila* AD brain, some of which were prevented by increasing Tip60 levels in the brain. Thus, we are the first to uncover distinct histone and RNA binding capabilities for Tip60 that mediate its function in

neural gene control and RNA splicing, respectively, and may underly the chromatin packaging and splicing defects that are now characterized as hallmarks of AD.

533A Temporal regulation of neuronal maturation by a chromatin anti-looping factor *Dahong Chen*, Catherine McManus, Behram Radmanesh, Leah Matzat, Elissa Lei NIH

The nervous system undergoes dramatic post-mitotic reorganization to become fully mature, but how chromatin 3D structure of neurons is regulated to assure proper transcriptomic dynamics during this essential process remains undefined in any organism. The tissue-specific chromatin insulator antagonist Alan Shepard (Shep) promotes *Drosophila* post-mitotic maturation by repressing expression of master regulator genes specifically in maturing neurons. To understand the mechanism of Shep repression of a key target gene, *brain tumor* (*brat*), we performed 4C-seq on CNS-derived BG3 cells and found that Shep depletion leads to increased *brat* promoter looping to proximal regions. Subsequent luciferase reporter assays verified enhancer activity of one of these candidate regions. Notably, depletion of Shep does not affect either enhancer or promoter activities in this artificial juxtaposed context, suggesting that Shep acts as a dedicated anti-looping factor to inhibit *brat* transcription. Interestingly, *in vivo* 3C also detected Shep inhibition of looping between the *brat* promoter and a proximal genomic region in pupal but not larval brains. Subsequent *in vivo* GFP reporter assays detected enhancer activity of this region specifically in pupal brains. This enhancer activity was not affected by Shep depletion, consistent with an anti-looping mechanism underlying Shep repression of *brat* transcription in pupal maturing neurons. Moreover, ATAC-seq and CUT&Tag for H3K4me1 on FACS-sorted neurons indicated that Shep is required to restrain chromatin accessibility of the *brat* enhancer as well as 1,126 other enhancers in pupal but not larval neurons, suggesting extensive Shep-mediated enhancer closure during neuronal maturation genome-wide. These enhancers are highly enriched for Shep-bound loci that are also transcriptionally inhibited by Shep, suggesting temporal Shep inhibition of enhancer accessibility to repress gene expression. Taken together, our results provide the first evidence for a chromatin anti-looping factor that regulates temporal enhancer accessibility and gene expression during organismal development.

534B Sex-specific variation in R-loop formation in *Drosophila melanogaster* *Timothy Stanek*[1,2], Rohan Mehra[1], Weihuan Cao[1], Christopher Ellison[1] 1) Department of Genetics, Rutgers University, Piscataway, NJ; 2) Department of Pathology, Robert Wood Johnson Medical School, Piscataway, NJ

R-loops are three-stranded nucleotide structures consisting of a DNA:RNA hybrid and a displaced ssDNA non-template strand. Originally viewed as byproducts of transcription, R-loops are now recognized as important regulators of gene expression and genomic stability. Persistent dysregulation of R-loop maintenance can result in replication stress, DNA double-strand breaks, and chromosomal rearrangements that contribute to diseases such as neurological disorders and cancer. Although R-loops are conserved across cell types in mammals, little is known about natural variation in R-loop formation between individuals.

Using DNA:RNA immunoprecipitation followed by high-throughput sequencing (DRIP-seq), we have mapped the R-loop profiles of two *D. melanogaster* individuals from the Drosophila Genetic Reference Panel (DGRP) in both males and females. R-loops are largely conserved across individuals and between sexes. Consistent with previous studies, R-loops are found at 5'UTRs of protein-coding genes and across many classes of noncoding RNA. R-loops are also enriched at Polycomb response elements and topologically associating domain boundaries. More broadly, R-loops are enriched in both the active RED and Polycomb Group BLUE chromatin states and depleted from GREEN heterochromatin and BLACK repressive states. Differential enrichment analysis reveals a small number of sex-specific R-loops: while non-differentially enriched and male-enriched R-loops form at similar genetic features and chromatin states and contain similar sequence motifs, female-enriched R-loops are enriched at unique genetic features, chromatin states, and sequence motifs. Male-enriched R-loops are most abundant on the dosage-compensated X chromosome, where R-loops appear stronger compared to autosomal R-loops. However, where most R-loop-containing genes exhibit higher levels of expression compared to R-loop-absent genes, male-enriched R-loops on the X chromosome do not correspond to increased gene expression. These male-enriched R-loops also show lower MOF binding and reduced H4K16ac, suggesting that canonical marks of hypertranscription associated with dosage compensation are antagonistic to R-loop formation. Collectively, these results reveal a series of sex-specific characteristics of R-loop formation in vivo and suggest that these hybrid structures can act to the benefit or detriment of specific cellular processes.

535C HDAC-inhibitory Microbial Volatiles Effect on Slowing Huntington's Disease in a Drosophila model *Rogelio Nunez Flores*, Sachiko Haga-Yamanaka, Christi Ann Scott, Anandasankar Ray University of California, Riverside

Eukaryotes coevolve with microbiomes and respond to their secreted metabolites. However, the response to volatile compounds emitted by microbes is poorly understood. We show that a microbial volatile, diacetyl, and other structurally related odorants, can alter gene expression in eukaryotes at a distance from their emission source. These compounds were found to inhibit human histone-deacetylases (HDACs) leading to increased histone-H3K9 acetylation. Most notable, these compounds inhibit HDAC6, a promising up-regulated target in several neurodegenerative diseases. These

epigenetic alterations explain the significant changes in gene expression/ physiological changes seen in Drosophila melanogaster models. In a Drosophila melanogaster transgenic model for Huntington's disease in the fly eye, exposure to the volatiles slowed progression of neurodegeneration similarly to known orally administered HDAC-inhibitor drugs. When we repeated the exposure study in HDAC6[KO] flies bearing the Huntington's Disease transgene, we also observed similar neuroprotective effects. Our findings suggest that these changes are caused by a conserved atypical signaling pathway that modulates gene expression via changes in chromatin from a distant chemical source.

536A Unique chromatin characteristics allow a genome-eliminating B chromosome to avoid self-elimination *Salina Teklay*[1], Haena Lee[1,3], Pooreum Seo[1], Emily Yuguchi[1], Elena Dalla Benetta[1,3], John Werren[2], Patrick Ferree[1] 1) Claremont Colleges, Claremont, CA; 2) University of Rochester, Rochester, NY; 3) UC San Diego, San Diego, CA

Insects harbor a wide array of reproductive parasites, including selfish B chromosomes. One of the most dramatic examples is a B chromosome known as PSR (for Paternal Sex Ratio), which resides in natural populations of the jewel wasp, *Nasonia vitripennis*. PSR is paternally transmitted via the sperm's nucleus to progeny, and it causes complete destruction of the sperm's hereditary material, but not itself, during the first embryonic mitotic division. This action converts female-destined embryos into haploid males, which can then transmit PSR. A compelling question is how PSR escapes its own genome-eliminating activity. We previously found that while PSR causes heightened H3K27me1 and H4K20me1 across the paternal genome just before it is eliminated, PSR itself does not incur these marks. Here we have found that the segregation of PSR is only partially affected by the CI activity of Wolbachia. While Wolbachia like PSR causes heightened H3K27me1 and H4K20mel across the paternal genome, PSR remains unmarked regarding these histone modifications in the presence of this chromatin-altering bacterium. These findings suggest that PSR's chromatin exhibits unique properties compared to the essential genome complement. We hypothesized that PSR may not participate in the histone-to-protamine transition as a way of evading chromatin disruption. However, this idea was not supported; when the histone chaperone HIRA was knocked down by systemic RNAi, PSR completely failed to segregate. Our current efforts are aimed at examining other chromatin-related characteristics to explain PSR's intriguing ability to escape its own genome eliminating activity.

537B Determining how H4K20 methylation contributes to L(3)mbt recruitment to chromatin *Megan B. Butler*, Aaron T. Crain, Robert J. Duronio University of North Carolina at Chapel Hill, Chapel Hill, NC

Generation and maintenance of specific chromatin domains are essential for proper genome regulation, cell cycle progression, and organismal development. Chromatin is a complex of DNA wound around histone proteins which have N-terminal tails that can be chemically modified post-translationally (PTMs). Proteins termed "readers" bind histone PTMs and either directly or as part of multi-protein complexes alter chromatin structure to control critical genome functions. One such reader discovered in *Drosophila melanogaster* and called Lethal (3) malignant brain tumor (L(3) mbt) functions as a tumor-suppressor in larval brains. L(3)mbt has been implicated in chromatin condensation and transcriptional regulation. Previous *in vitro* studies showed that L(3)mbt and its human homolog preferentially bind histone H4 that is methylated on lysine 20 (H4K20me). However, *in vivo* studies have shown that L(3)mbt does not exclusively co-localize with H4K20me throughout the genome; thus, how L(3)mbt interacts with chromatin and exerts its functions 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. With our histone gene replacement platform in *Drosophila,* we generated H4K20A and H4K20R mutants to assess the consequence of eliminating H4K20me on L(3)mbt interaction with the genome. In order to perform this experiment, we built GFP- and FLAG-tagged alleles of *l(3)mbt* at the endogenous locus using CRISPR-Cas9 and the Scarless Gene Editing system. These alleles complement a null mutation of *l(3)mbt*, indicating that the epitope-tagged proteins are fully functional. We assessed L(3)mbt's cytological localization in wildtype and H4K20-mutant animals via confocal microscopy using anti-GFP and anti-FLAG antibodies. In wildtype larval brains, imaginal discs, and adult ovaries, L(3)mbt is predominantly nuclear during interphase with much of the protein residing in the nucleoplasmic compartment. During mitosis, L(3) mbt disperses throughout the cell after nuclear envelope breakdown and does not localize to condensed mitotic chromosomes. In H4K20-mutant animals, we detected a small decrease in total L(3)mbt signal without any change in L(3)mbt localization. These results indicate that we are unable to use cytology alone to make conclusions about whether H4K20me is necessary for L(3)mbt chromatin binding. Therefore, we will next use approaches like CUT&RUN to determine the association of these epitope-tagged L(3)mbt proteins with the genome in wildtype and H4K20-mutant animals at a high resolution.

538C Interrogating the roles of canonical versus variant histone H3 in genome function and aging *Jeanne-Marie McPherson*, Robert Duronio, Daniel McKay University of North Carolina at Chapel Hill

Histones are essential for packaging and organizing DNA into chromatin, which regulates all DNA-dependent processes. Regulation of histone abundance is critical, as too many or too few histones is toxic to cells and disrupts development. Cells contain two types of histones: canonical histones expressed during S phase of the cell cycle and variant histones expressed throughout the cell cycle and in post-mitotic cells. Canonical histone H3.2 and variant histone H3.3 are highly

conserved across eukaryotes, making *Drosophila melanogaster* an ideal model to study histone biology. By manipulating canonical H3.2 copy number in *Drosophila* we discovered that H3.3 is essential for development when canonical histone gene copy number is reduced, uncovering a previously unknown requirement for coordination between canonical H3.2 and variant H3.3 expression. We hypothesize that cells possess a homeostatic mechanism to maintain the correct relative expression of canonical H3.2 and variant H3.3. Histone homeostatic mechanisms could affect histone expression at many levels, including transcription, translation, deposition, or protein turnover. Thus far we have discovered that reducing canonical H3.2 copy number does not result in increased variant H3.3 transcript or protein levels. We will explore the mechanisms of histone homeostasis by conducting a forward genetic screen to identify factors involved in canonical and variant histone coordination. This project expands our fundamental understanding of how canonical and variant histones cooperate to regulate genome function.

539A A novel mosaic system for performing forward genetics in a sensitized histone mutant background *Aaron T. Crain*, Robert J. Duronio University of North Carolina at Chapel Hill, Chapel Hill, NC

The mono-methylation of lysine 20 of histone H4 (H4K20me1) is involved in numerous processes necessary for cell proliferation, including DNA replication, gene expression, and chromosome condensation during mitosis. These functions of H4K20me1 have been largely derived from interpreting the phenotypes resulting from manipulation of the activity of Set8, the lysine methyltransferase responsible for depositing H4K20me1. However, such interpretations are complicated by studies demonstrating that Set8 has several non-histone substrates with essential roles in cell proliferation, such as p53 and PCNA, as well as non-catalytic functions. To directly assess the contribution of H4K20me in cell proliferation, we engineered a novel system for generating mosaic tissues containing clones of histone mutant cells. We first created a new, precise deletion of the replication dependent histone locus on chromosome 2 (*HisC*) that is marked with *Act5c-dsRed*. This allele was then combined with an established transgenic histone replacement platform in which a transgene containing a synthetic histone gene array capable of complementing homozygous *HisC* deletions is integrated on chromosome 3. These transgenes are engineered to express either wild type (WT) or mutant (e.g. H4K20A or H4K20R) histones. We then use FLP/FRT-mediated mitotic recombination to produce dual-color mosaic tissue in the *Drosophila* eye, where one color (green) labels cells expressing wild-type histones and the other color (red) labels cells expressing only mutant histones. Thus, we can easily visualize and quantify competitive proliferation of histone mutant cells adjacent to wild-type cells. Strikingly, we found that both $H4^{K20A}$ and $H4^{K20R}$ mutant cells can proliferate adjacent to wild-type cells, achieving 34% and 22% of the adult eye, respectively, compared to 42% for $H4^{WT}$. In contrast, we found that $Set8^{null}$ mutant cells cannot proliferate adjacent to wild-type cells and die. We interpret the lack of proliferation and death of $Set8^{null}$ cells as likely due to H4K20me-independent mechanisms, and thus conclude that the role of H4K20me in cell proliferation remains unknown. To discover the functions of H4K20me in cell proliferation we will utilize our newly established mosaic system to perform a forward genetic screen for modifiers of proliferation of H4K20 mutant cells. This work establishes the first platform for performing forward genetics using histone mutant cells in animals.

540B Identification of factors involved in rDNA magnification in the male germline *Alyssa Slicko*[1,2], Jonathan Nelson[1], Yukiko Yamashita[1,2,3] 1) Whitehead Institute, Cambridge, MA; 2) Howard Hughes Medical Institute; 3) Department of Biology, MIT, Cambridge, MA

An important role of the germline is to maintain unstable, but essential genomic elements to ensure the transmission of functional genomes across generations. Among these elements are ribosomal DNA (rDNA), which consists of hundreds of tandem repeats of the genes that are needed for sufficient ribosomal activity. rDNA is known to be one of the most unstable regions in the genome because this repetitive structure is susceptible to deleterious recombination. This instability is countered in the germline to prevent the progressive multi-generational loss of rDNA, but the mechanisms that underlie this feature have remained unknown. Our previous work suggests that rDNA is maintained by copy number expansion in the germline that recovers copies lost in the previous generation, though the factors that achieve this expansion remain largely unknown. Here we describe our efforts to identify these factors through RNAi-mediated knockdown of candidate genes.

We used a well-described system of robust rDNA copy number expansion in the male germline called “rDNA magnification” to identify factors required for rDNA expansion. Previous work revealed that animals with very little rDNA, meaning no rDNA on Y and partial rDNA on X, have the bobbed phenotype, which displays disrupted dorsal cuticle patterning. rDNA magnification is the phenomenon that a small amount of offspring from bobbed males have wild-type cuticles due to the rDNA on the partially deleted X chromosome having been expanded. We tested the ability for RNAi knockdown of candidate genes to prevent rDNA magnification in bobbed males.

We selected candidate factors of known rDNA-binding proteins, and homologs of factors that aid in rDNA maintenance in yeast, to test with the RNAi. We preliminarily identified that an uncharacterized gene on the Y chromosome, PRY (Polycystine-related Y), contributes to rDNA magnification. PRY was tested as a candidate factor since it resides in

a portion of the Y chromosome previously identified to be necessary for rDNA magnification. We discovered that the transgenic expression of PRY can induce rDNA magnification in both the male and female germline. We are now investigating the activity of PRY in the germline to understand how it contributes to rDNA magnification.

541C Genomic insertion of repetitive DNA can trigger conversion of euchromatin to heterochromatin *Safiyo Aden*, Derrick carper, Heidi J.J Pipkin, Andrew M Arsham Bemidji State University

Heterochromatin is a key genomic defense against invasive genetic elements, mitigating damage by inhibiting transposition, silencing gene expression, and reducing recombination at insertion sites. How genomes recognize and silence novel threats prior to establishing sequence-specific adaptive defenses like piRNAs is poorly understood. To investigate genome defense against novel repetitive DNA we carried out a transposition mutagenesis screen with a reporter construct expressing the white gene adjacent to a 256-copy tandem array of a 36 nt lac operator sequence from E. coli. Reporter gene expression was robust in the vast majority of transposition mutants recovered, while approximately 1% had variegated eye color suggesting epigenetic silencing by heterochromatin. Mapping of insertion sites by inverse PCR revealed that the lacO reporter construct can trigger silencing of actively transcribed euchromatin. Excision of the lacO array by FLP-FRT recombination suppressed variegation, restoring full reporter gene expression and demonstrating repeat-dependent silencing. Ectopic heterochromatinization a lacO tandem array may model disease processes like the silencing of triplet nucleotide expansions and evolutionary processes like centromere formation.

542A Role of Ulp1, a SUMO E3 Protease, in enabling 'safe' Homologous Recombination progression at the nuclear periphery. *Nadejda Butova*, Irene Chiolo, Chiara Merigliano, Taehyun Ryu, Sydney Prange University of Southern California

Pericentromeric Heterochromatin occupies ~30% of fly and human genomes and is mostly composed of highly repeated DNA sequences prone to aberrant recombination. And yet, repair mechanisms in heterochromatin remain poorly understood. Previous studies from the Chiolo lab in Drosophila cells showed that 'safe' homologous recombination (HR) repair of heterochromatic double-strand breaks (DSBs) requires the movement of repair sites away from the heterochromatin 'domain' (a distinct nuclear structure in fly cells) to the nuclear periphery, where the strand invasion protein Rad51 is recruited to continue repair. This is believed to prevent aberrant recombination by isolating broken sites and their homologous templates away from ectopic (non-allelic) repair templates before strand invasion. The post-translational modification SUMOylation is essential for this pathway, as it blocks Rad51 recruitment and HR progression inside the heterochromatin domain thus preventing aberrant recombination. Furthermore, work in the Chiolo lab has shown that Ulp1 overexpression leads to abnormal Rad51 recruitment to repair sites inside the HC domain. What restarts HR at the nuclear periphery, the SUMOylation targets, and the role of SUMOylation in repair are major open questions in the field. By identifying these targets and their role in repair of ionizing radiation (IR)-induced DSBs, our studies will unravel new molecular mechanisms preventing chromosome rearrangements and genome instability in heterochromatin, which are still obscure, as well as establish the role of Ulp1 in the spatial and temporal regulation of heterochromatin repair. Through the use of Mtor/Tpr depletions and Ulp1ΔN mutants that lose the nuclear-pore association domain, thus releasing Ulp1 in the nucleoplasm, my results show how Mtor/Tpr is required to recruit Ulp1 to the nuclear periphery where it is able to complete repair. By testing the role of Ulp1 at the nuclear periphery using Ulp1 depletions by RNAi or degrons, the loss of Rad51 foci at heterochromatic DSBs (marked by γH2Av foci associated with heterochromatin marks), at the nuclear periphery in the absence of Ulp1 was observed. This supported the role for Ulp1 in HR progression once DSBs have relocalized. Overall, these results support the hypothesis that Ulp1 compartmentalization to the nuclear periphery is necessary for preventing abnormal HR progression inside the heterochromatin domain, while enabling 'safe' HR progression at the nuclear periphery.

543B Investigating the origin and evolution of CG17359, rapid-evolving, essential ZAD-ZNF gene in multiple *Drosophila* species *Madeline Gruys*[1, 2], Zainab Abdulrahman[1, 2], Ashlyn Anderson[1, 2], Safiyo Aden[1, 2], Solomon Aviles[1, 2], Derrick Carper[1, 2], Nathan Dupre[1, 2], Jack Jurmu[1, 2], Tetiana Khotko[1, 2], Zahraa Lami[1, 2], Hunter Lindsay[1, 2], Tea Merkl[1, 2], Heidi J. J. Pipkin[1, 2], Dennis Quach[1, 2], Anthony Ruiz[1, 2], Melissa K. Sawyer[1, 2], Noah Stone[1, 2], Bailey Taylor[1, 2], Barbara Whitlock[1, 2], Christina Yang[1, 2], Emily Yang[1, 2], Andrew M Arsham[1] 1) Bemidji State University; 2) North Hennepin Community College

Ancient essential genes are highly conserved due to strict functional constraints that limit their evolutionary potential. It has become clear that young and rapidly evolving genes can also acquire new functions and become essential. The evolution of some of these genes may be driven by the dynamic nature of heterochromatin and to maintain silencing of rapidly-changing non-coding heterochromatic sequences. The evolutionarily dynamic ZAD-ZNF gene family encodes the most abundant class of insect transcription factors, many of which appear to be the product of relatively recent segmental duplication or retrotransposition. CG17359 and CG17361 are adjacent paralogous ZAD-ZNF genes that appear to have formed by retrotransposition between 30 and 40 million years ago. CG17359 is essential in D. melanogaster and shows evidence of positive selection, while CG17361 is not essential and does not show evidence of strong selective

pressure. We have identified a third nearby ZAD-ZNF gene, CG8474/Meics, as a candidate ancestor for both. Using the gene annotation framework of the Genomics Education Partnership, we annotated sequence conservation and synteny for all 3 genes across Drosophila species spanning 40 million years of evolution. The quickly-evolving yet essential members of the ZAD-ZNF gene family suggest candidate heterochromatin regulatory genes for functional testing.

544C Towards telomere-to-telomere genome assemblies of *Drosophila melanogaster* Nicolas Altemose[1], Susan E. Celniker[2], Mahul Chakraborty[3], Cécile Courret[4], *J.J. Emerson*[3], Gary H. Karpen[1,2], Bernard Y. Kim[5], Charles H. Langley[6], Sasha Langley[1], Amanda M. Larracuente[4], Barbara Mellone[7], Karen H. Miga[8], Danny E. Miller[9,10], Rachel J. O'Neill[7], Adam M. Phillippy[11], Brandon D. Pickett[11], Harsh G. Shukla[3], The Drosophila Telomere-to-Telomere Consortium 1) University of California, Berkeley, Berkeley, CA; 2) Lawrence Berkeley National Lab, Berkeley, CA; 3) University of California, Irvine, Irvine, CA; 4) University of Rochester, Rochester, NY; 5) Stanford University, Stanford, CA; 6) University of California, Davis, Davis, CA; 7) University of Connecticut, Storrs, CT; 8) University of California, Santa Cruz, CA; 9) University of Washington, Seattle, WA; 10) Seattle Children's Hospital, Seattle, WA; 11) National Human Genome Research Institute, NIH, Bethesda, MD

The goal of genome sequencing and assembly is to capture all sequence features that play critical roles in organisms and to join them together as parts of complete, gapless chromosome reference assemblies. However, in *Drosophila melanogaster*, the most complete assemblies are at least 15-20% smaller than the known genome size. The segments missing from assemblies are almost entirely composed of repetitive sequences, primarily transposable elements and satellite repeats concentrated in pericentromeric heterochromatin. These missing regions not only contain essential genes, they also harbor other elements that play crucial roles in genome stability, chromosome segregation, protein translation, and TE repression. To recover these important regions, we are building telomere-to-telomere assemblies of multiple *D. melanogaster* strains, including the genome reference strain iso-1. Our goal is to produce complete, extremely accurate (fewer than 1 error per megabase) assemblies for all three autosomes and the X and Y chromosomes. Efforts by the human Telomere-to-Telomere (T2T) Consortium have pioneered the completion of virtually gapless chromosome assemblies that span large repetitive arrays, including satellites, scrambled transposable elements, and ribosomal DNAs. These approaches leverage ultra-long sequencing reads to untangle assembly graphs derived from highly accurate long sequence reads. By applying this approach to additional strains, we can study variation in chromosome structures that have previously resisted scrutiny, like centromeres. This open, collaborative initiative aims to produce a gapless assembly of *D. melanogaster*, outline best practices for extending this approach to other strains and species, and support public accessibility of data releases and methodologies.

545A Silencing and position-effect variegation in a dual-reporter transposition mutagenesis screen *Nathan Dupre*[1,2], Shyanne Abbott[1,2], Safiyo Aden[1,2], Ashlyn Anderson[1,2], Solomon Aviles[1,2], Deneisha Bergquist[1,2], Derrick Carper[1,2], Rhianna Coffey[1,2], Max Her[1,2], Clara Hovland[1,2], Marayan Ibrahim[1,2], Hunter Lindsay[1,2], Heidi Pipkin[1,2], Kyle Reichstadt[1,2], Anthony Ruiz[1,2], Melissa Sawyer[1,2], Christina Yang[1,2], Emily Yang[1,2], Andrew Arsham [1,2] 1) Bemidji State University; 2) North Hennepin Community College

Highly repetitive DNA sequences are often associated with invasive or pathogenic DNA, and can also be found in gene-poor regions of eukaryotic genomes like centromeres and telomeres. Genomes often defensively package repeats in dense, transcriptionally refractory, stably heritable heterochromatin which plays a crucial role in the regulation of gene expression through transcriptional silencing. How these sequences trigger the establishment and maintenance of heterochromatin remains poorly understood, but repeats appear to serve as a signal of foreignness to host genomes and can activate the siRNA, miRNA, or piRNA silencing pathways. Silencing of repetitive DNA is implicated in the formation and function of centromeres and telomeres, in defense against transposons and viruses, and in human trinucleotide repeat expansion diseases like Friedreichs Ataxia and Fragile X syndrome. To investigate the mechanism and regulation of repeat-triggered silencing, undergraduate students participating in a classroom research experience (CURE) carried out a transposition mutagenesis to investigate the effects of large tracts of repetitive DNA on gene expression and chromatin state. Students mobilized a p-element containing a 256-copy tandem array of the *E. coli* lac operator (LacO), flanked by *white* and *yellow* reporter genes. After setting up mobilization crosses, students screened F2 progeny for changes in eye or body color indicating expression of either or both reporter genes (287/14137 or 2% of all male F2 screened). Of transgene-expressing flies, wild type expression of yellow and white comprised 82% of all mutants and indicate presumed euchromatic insertions resulting in high reporter gene expression. The *white* gene alone was silenced in 11% of identified mutants; the *yellow* gene alone was silenced in 4%, demonstrating a surprising decoupling of reporter gene silencing. 3% of mutants showed variegation of the *white* gene and full expression of the *yellow* gene, but the converse was not observed, nor was variegation of body color. Molecular mapping of insertion sites with inverse PCR suggest that LacO repeat insertion can trigger silencing even in gene-rich euchromatic locations.

546B The Phosphorylated Histone Variant H2Av Associates With *Gypsy* Insulator Proteins Through Liquid-Liquid Phase Separation James Simmons, Ran AnAndrea , Bright Amankwaa, Shannon Stroud, Andrea Mancheno Lopez , *Mariano Labrador* The University of Tennessee at Knoxville

Eukaryotic genomes are characterized by a highly orchestrated 3D organization. Binding of insulator proteins to specific DNA sequences through the genome contribute to genome structure by demarcating the boundaries between genome domains. The functional properties of insulator proteins, along with those of other architectural proteins, are under strong scrutiny given that the understanding of genome structure is essential to explain fundamental aspects of genome expression and genome maintenance. Loop extrusion mediated by Cohesin and chromatin-mediated liquid-liquid phase separation (LLPS) are thought to be the major driving forces contributing to domain formation and compartmentalization of the genome. The *Drosophila melanogaster* genome expresses an array of insulator proteins that constitute diverse insulator complexes targeted to different sequences across the genome, but how these insulator complexes specifically contribute to genome structure is currently unknown. Although insulator proteins function in the context of chromatin and nucleosomes, specific histone proteins have never been directly implicated in insulator function. Here, we demonstrate an interaction between *gypsy* insulator proteins and the phosphorylated form of the histone variant H2Av (γH2Av). We show that components of the gypsy insulator complex, Su(Hw), Mod(mdg4)67.2 and CP190 colocalize with γH2Av throughout the genome and mutation of insulator components prevents stable association of phosphorylated H2Av with chromosomes. Inhibition of the PP2A phosphatase results in a stronger chromosomal association of γH2Av and insulator proteins. Interestingly, γH2Av but not unphosphorylated H2Av associates to insulator bodies after osmotic stress, and phosphatase activity is required for insulator body dissolution after recovery from stress. Our evidence suggests a model in which phosphorylation of H2Av regulates insulator activity by modulating the LLPS properties of insulators and associated architectural proteins.

547C Cardiac aging prevention through H3K27me3 modulation *Clara Guida*, Georg Vogler, Peter Adams, Rolf Bodmer Sanford Burnham Prebys Medical Discovery Institute

The incidence of heart failure approximately doubles through each decade of life. The detrimental effects of age on heart function are likely due in part to epigenetic dysregulation and associated gene program changes. Indeed, evidence already suggests that the maintenance of the epigenome becomes more error-prone with age, leading to so-called “epigenetic drift”, or accumulation of epigenetic alterations. Interestingly, manipulation of certain epigenetic modifiers was found to expand lifespan in different animal models. However, little is known about the epigenetic mechanism underlying cardiac aging. The challenge of studying cardiac aging relies in part on the low abundance of human samples and long and expensive experimental times for mammalian animal models. Here we used the *Drosophila* heart model, which has several advantages including short lifespan, less genetic redundancy, conserved biological pathways, and *in vivo* heart analysis protocols, to identify epigenetic mechanisms involved in cardiac aging. In a targeted genetic screen, we found that partial depletion of components of the Polycomb Repressive Complex 2 (PRC2) prevented cardiac aging. This is in line with previous findings reporting increased lifespan in $PRC2^{+/-}$ flies. Indeed, we found that H3K27me3 content is increased in cardiomyocytes with age in control flies. Moreover, age-related cardioprotection was also achieved when we treated flies with an inhibitor of the H3K27me3 methyltransferase, EZH2. These findings suggest that H3K27me3 marks can mediate age-related heart function deterioration and establish the *Drosophila* model as a useful tool to investigate the basic biology of age-related cardiac epigenetic drift.

548A Investigating the role of Polycomb repression in *Drosophila* eye specification *Haley Brown*, Justin Kumar Indiana University, Bloomington, IN

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* mutant EADs throughout third instar development. This analysis identified 55 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. 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. I will further investigate how repressive and active histone modifications are changing in this system to allow for reprogramming by using a novel epigenome profiling technique, CUT&RUN. I hypothesize that the inability of *Pc* mutants to read H3K27me3 marks allows the epigenome to become malleable, activates wing determination genes, and transforms the eye into a wing. Outcomes of this study will elucidate the mechanistic role

epigenetic factors play in tissue fate determination, ultimately providing insight into how mutations in epigenetic proteins result in human developmental disorders.

549B The H3.3K27M oncohistone antagonizes reprogramming in Drosophila *Kami Ahmad*[1], James Anderson[1], Steven Henikoff[1,2] 1) Fred Hutchinson Cancer Research Center; 2) Howard Hughes Medical Institute

Development proceeds by the activation of genes by transcription factors and the inactivation of others by chromatin-mediated gene silencing. In certain cases development can be reversed or redirected by mis-expression of master regulator transcription factors; this reprogramming must involve the activation of new genes and the chromatin-mediated silencing of others. Here, we express the wing-specific Vestigial master regulator to reprogram the developing eye, and test the role of silencing in reprogramming using an H3.3K27M oncohistone mutation that dominantly inhibits histone H3K27 trimethylation. We find that production of the oncohistone blocks eye-to-wing reprogramming. CUT&Tag chromatin profiling of mutant tissues shows that H3K27me3 of domains is generally reduced upon oncohistone production, suggesting that a previous developmental program must be silenced for effective transformation. Strikingly, Vg and H3.3K27M synergize to stimulate overgrowth of eye tissue, a phenotype that resembles that of the oncohistone in human cancers. Transcriptome profiling of elongating RNA Polymerase II implicates the mis-regulation of signaling factors in overgrowth. Our results demonstrate that growth dysregulation can result from the simple combination of crippled silencing and transcription factor mis-expression, an effect that may explain the origins of oncohistone-bearing cancers. We have continued this work to search for additional factors that can synergize with the H3.3K27M oncohistone, and to probe the earliest epigenetic changes in cells as they begin to overproliferate.

550C X marks the spot: Specifically targeting active chromatin to the X chromosome *Joseph Aguilera*, Mukulika Ray, Ashley Conard, Erica Larschan 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. ***Chromatin domains*** coordinate gene expression by concentrating key factors at specific genomic locations in a context-specific way to concordantly activate or repress groups of genes. Before zygotic genome activation (ZGA), chromatin domains have not yet formed and only a few key transcription factors (TFs) are bound which are called ***pioneer TFs***. Pioneer TFs have the ability to bind to closed chromatin, recruit chromatin remodelers to open chromatin, and target additional transcription complexes. Furthermore, pioneer TFs are critical for cellular reprogramming and are often reactivated in cancer. Pioneer TFs can regulate the formation of active and repressive chromatin domains which form after ZGA. *However, the fundamental mechanisms by which chromatin domains are formed at the correct genomic locations across time and space remain unknown.* One example of a conserved active chromatin domain is the hyperactivated single male X-chromosome in Drosophila, which coordinately upregulates thousands of genes two-fold to equalize transcript levels with the two autosomes. My preliminary data suggest that the interaction between an essential pioneer TF and large transcription complex is important to target this active chromatin domain specifically to the X-chromosome. Therefore, I will use genomic, optogenetic, and machine learning approaches to define new mechanisms by which a pioneer TF nucleates the formation of an active chromatin domain that upregulates the male X-chromosome over time and space.

551A Intercalary heterochromatin prevents local somatic pairing loss in interspecies Drosophila hybrids *James Baldwin-Brown*, Nitin Phadnis University of Utah

Homologous chromosome pairing is essential to all eukaryotes. Even so, our understanding of the molecular mechanisms underlying pairing is limited. We used changes in the pattern of pairing in Drosophila hybrids to find new mechanisms connected to pairing, and find that BLACK (intercalary) heterochromatin correlates with successful pairing. This discovery will help us understand pairing generally.
Although pairing is often associated with meiosis, it also occurs in somatic cells. Complete somatic pairing is the wild type state in Drosophila and other dipterans, but reproducible patterns of unpaired regions exist across the genome in interspecific Drosophila hybrids. Because chromosome pairing machinery exists in all eukaryotes, finding the genomic elements that drive this non-pairing will help us understand the drivers of pairing generally.
We crossed Drosophila melanogaster and Drosophila simulans, then used Hi-C to measure the rate of chromosome pairing with high resolution across the genome. Compared to within-species crosses, this hybrid Hi-C shows dramatic regions of high and low pairing. Repeating experiments in multiple tissues showed that hybrid pairing loss uniquely affects polytene tissues. In keeping with results from mosquitoes, an inversion between D. melanogaster and D. simulans showed reduced pairing around the inversion breakpoints, serving as a positive control.
While past microscopy experiments showed the existence of non-pairing regions, our high-resolution methods show a complex tapestry of high- and low- pairing regions of varying breadths and intensities. The clearly resolved peaks are consistent with the “button” model of pairing (multiple, discrete pairing sites) rather than the “zipper” model (continuous pairing along the chromosomes). We showed that local pairing maxima and minima are apparently uncorrelated with expected pairing drivers such as sequence similarity and insulator binding site density. High pairing is, however, highly correlated with BLACK chromatin. This chromatin is underreplicated in tissues with multiply replicated

genomes such as the polytene tissue we measured. We hypothesize that replication machinery is slowed by BLACK chromatin, and helps maintain pairing when other pairing influencers are non-functional. Future work will test this underreplication hypothesis and show whether genes that influence reproductive incompatibility between these species also drive hybrid pairing loss.

552B A telomere associated system of paramutation in *Drosophila virilis* mediated by maternally provisioned piRNAs *Ana Dorador*, Justin Blumenstiel University of Kansas

Paramutation is the phenomenon by which a silent alle can turn off a normal allele in *trans* in an epigenetic manner. The silenced state of the wildtype allele can persist through generations even in the absence of the original paramutagenic allele. The mechanisms underlying paramutation are poorly understood. Further, little is known about how paramutation shapes gene expression under natural conditions. In this study, we investigate a system of genic paramutation in *Drosophila virilis*. Previous studies have shown that maternally transmitted piRNAs that target the *center divider* (*cdi*) gene in *D. virilis* have the capacity to silence expression of *cdi* in the next generation. In addition, it has been shown that piRNAs that target *cdi* can be maintained in subsequent generations in the absence of the original silencing allele. However, it is not known whether this pattern of piRNA biogenesis and maternal transmission coincides with epigenetic repression of *cdi* expression across multiple generations. To determine if *cdi* piRNA biogenesis mediates paramutation, we measured the expression of *cdi* in the ovaries of females heterozygous for the silencing allele, as well as their daughters that lack the silencing allele.
In two independent experiments, *cdi* expression was quantified in 20 F1 heterozygous mothers and 20 first-generation backcross daughters, lacking the original silent allele, using RT-qPCR. Confirming previous studies, we found that heterozygous females that maternally inherited the piRNA producing allele had low expression of *cdi* in ovaries. We further found that the first-generation backcross daughters - lacking the paramutagenic allele – had significantly lower *cdi*expression in the ovaries compared to a genotypically identical strain with no piRNAs mapping to *cdi.* This study thus describes a new system of paramuation in gene expression of *Drosophila virilis*, which can serve as a baseline for future studies that seek to understand how paramutation is regulated.

553C Activating and repressing stochastic gene expression between chromosomes *Elizabeth Urban*, Chaim Chernoff, Kayla Viets, Jeong Han, Caitlin Anderson, Sang Tran, Daniel Konzman, Robert Johnston Johns Hopkins University, Baltimore MD

DNA elements act across long genomic distances to regulate gene expression in processes including enhancer-promoter interactions and genetic imprinting. During the gene-regulatory phenomenon of transvection in *Drosophila*, DNA elements on one allele of a gene act between chromosomes to increase or decrease expression of another allele of the gene. Despite the discovery of transvection over 60 years ago, little is known about its biological role. Furthermore, how separable DNA elements contribute to activating or repressing transvection at distinct times during development is unclear. Here, we study the expression of *spineless* (*ss*) in the developing fly eye as a paradigm to understand gene activation and repression between chromosomes. We found a biological role for transvection in controlling the stochastic expression of naturally occurring *ss* alleles. We characterized CRISPR engineered deletions of sequences across the *ss* locus and identified DNA elements required for activating and repressing transvection. We found that enhancers participate in transvection at distinct times in development to promote gene expression. Finally, bringing a silencer element on a different chromosome into proximity with the *ss* locus “reconstitutes” the gene, leading to repression. Our studies show that transvection regulates gene expression via distinct DNA elements at specific timepoints in development with implications for genome architecture.

554A Investigating dBRWD3’s regulation on ORC by ubiquitination *Dongsheng Han*[1], Tara O’shea[1], Jonathan P Davies[2], Logan Richards[1], Lars Plate[2], Jared T Nordman[1] 1) Department of Biological Sciences, Vanderbilt University, Nashville, TN; 2) Department of Chemstry, Vanderbilt University, Nashville, TN

Posttranslational modifications of histones impact chromatin structure and function, which underlie everything from genome stability to cell differentiation and disease. Understanding how histone modifications are established and maintained is critical to understanding these processes. BRWD3 (Bromodomain and WD repeat-containing protein 3) is a specificity factor for a CUL4 ubiquitin ligase complex. BRWD3 has been implicated in DNA replication, transcription, and regulation of histone H3 methylation and acetylation. The underlying mechanisms of BRWD3 action, however, are largely unknown. To uncover how BRWD3 functions, we performed both dBRWD3 IP-MS and BRWD3-dependent ubiquitination IP-MS. As expected, we identified the BRWD3-Cul4-DDB1 E3 ubiquitination complex and multiple histones as significantly enriched in our dBRWD3-IP. Interestingly, we identified the Origin Recognition Complex (ORC) as both associated with BRWD3 and ubiquitinated in a BRWD3-dependent manner. Bioinformatic analysis revealed that ~35% of ORC2 binding sites overlap with dBRWD3 binding sites throughout the genome. Furthermore, depletion of dBRWD3 significantly reduces DNA proliferation in the S phase. In addition to ORC, we have also identified several key chromatin-related proteins that could provide mechanistic insight into BRWD3 function. In conclusion, our approach has successfully identified likely direct targets of the BRWD3-Cul4-DDB1 E3 ubiquitin ligase complex, which could help explain how

BRWD3 functions in diverse chromatin-related processes.

555B Y2H screening reveals potential interactors of a B chromosome-expressed toxin in the jewel wasp *Isabella Draper*, Sammy Lee, Max Richmond, Patrick Ferree Claremont Colleges, Claremont, CA

Thousands of plants and animals are known to contain parasitic B chromosomes, many which drive, or segregate at super-Mendelian frequencies. An intriguing question is how B chromosome drive occurs at the mechanistic level. Recently we discovered a toxin gene, named *haploidizer*, which is expressed by the Paternal Sex Ratio (PSR) chromosome in the jewel wasp, *Nasonia vitripennis*. The presence of PSR causes complete elimination of the paternally inherited half of the wasp's genome, an action that converts female-destined embryos into males, the PSR-transmitting sex. Reduction of *haploidizer* transcripts in the testis by RNAi caused strong suppression of PSR's genome eliminating activity, demonstrating that this gene is essential in this function. To begin to understand the role of *haploidizer*, we performed yeast 2-hybrid (Y2H) screening with the putatively encoded *HAPLOIDIZER* protein. In the relatively small group of potential interactors, we found several proteins that may perform chromatin-associated functions. These findings are consistent with the hypothesis that PSR disrupts some aspect of paternal chromatin remodeling following sperm entry into the egg. Our group is currently using RNAi to test these candidate *HAPLOIDIZER* interactors.

556C DNA methylation machinery is required for transcriptome regulation and early development in the wasp Nasonia *Jeremy Lynch*[1], Deanna Arsala[1,2] 1) University of Illinois at Chicago; 2) University of Chicago

While DNA methylation is present in most insects, the toolkit has been lost in the lineage leading to Drosophila. Previous experiments in the wasp Nasonia vitripennis showed that the methylation toolkit component DNA methyltransferase 1 (Nv-dnmt1a) plays an important role in early embryogenesis, and we are using this model to understand the ancestral role of DNA methylation in insect embryos, and how it was replaced in species where the toolkit has been lost. We found that embryonic lethality of Nv-dnmt1a knockdown is preceded by scattered failures of blastoderm cellularization, and subsequent failures of morphogenetic movements. Such phenotypes are typical of defects in the maternal-zygotic transition (MZT), indicating that DNA methylation may have a role in regulating this process in the wasp. Using whole genome bisulfite sequencing, we show that knockdown of Nv-dnmt1a leads to strong reduction of gene body methylation throughout the genome. Using RNAseq, we show that ~90% of genes downregulated after Nv-dnmt1a RNAi are methylated (in wild-type embryos). This is not unexpected, as it has been previously shown that insect DNA methylation is associated with efficient transcription (and not repression or imprinting as found in vertebrate models). As development proceeds, more and more non-methylated loci are affected, showing a likely indirect effect of DNA methylation on the regulation of transcripts during the MZT. We propose that moderate disruption of the large number of methylated genes has cascading effects on the MZT that lead to failure of proper maternal clearance and/or zygotic activation of genes in early embryogenesis of Nasonia.

557A The histone chaperone NASP has multiple functions during development. *Reyhaneh Tirgar*, Shannon Leahy, Kendal Broadie, Jared Nordman Vanderbilt University, Nashville, TN

Histone chaperones aid in the disassembly and reassembly of nucleosomes during the processes of replication and transcription. While histone chaperones have been studied extensively at the molecular level, there remain more open questions about understanding the broader role histone chaperones play during development and disease conditions. Our focus is on the Nuclear Autoantigenic Sperm Protein (NASP) chaperones for histones H1 and H3/H4. These chaperones are required for proper DNA replication, cell cycle progression and cell proliferation. In mammals, NASP is homozygous lethal, but the contributions NASP makes during development are largely unknown. To understand the consequence of NASP loss on development, we used CRISPR/Cas9 to generate a NASP null mutant in *Drosophila.* Zygotic NASP is not essential for viability, however, loss of NASP function results in female sterility. This suggests that maternally deposited NASP has a critical function during embryogenesis. Further, NASP null mutants display severe movement impairments. This striking lack of mobility suggests neurological or neuromuscular defects, which we are currently investigating.

558B Environmental Effects on the Epigenetic Silencing of Transposable Elements Jennifer McIntyre University of California Irvine

Rapidly changing dietary and environmental conditions are of concern as to how they influence our genome. Transposable elements (TEs) are widespread DNA sequences that are able to move throughout the genome. They have large potentials to impact our genome, including perturbing the expression of neighboring genes via epigenetic mechanisms. Alzheimer's, muscular dystrophy, predisposition to cancer, and other diseases are often caused by TEs. Accordingly, it is important to determine how our environment affects this jumping DNA. To mitigate the spreading of TEs, repressive markers are enriched at the TE location. While this epigenetic marker is successful at limiting the movement of TEs, it can also repress neighboring genes. In this experiment, we used a reporter assay that consists of a reporter gene next to a TE. Altered expression level of the reporter gene would inform the extent of TE-mediated

silencing of neighboring reporter gene. We focus on two major directions: varied climate variables and altered dietary conditions. Our preliminary results suggest that, while changing climate conditions have inconclusive effects on repressive markers, altered diet is likely to have significant influences. We have already seen that a high sugar diet causes increased expression of the reporter gene This indicates reduced TE-mediated silencing of neighboring genes. We will continue testing other dietary conditions to identify factors that alteredTE-mediated silencing. Identification of these prevalent factors could help mitigate TE-mediated abnormal expression of important genes that cause detrimental diseases.

559V The detachment of lamin Dm0 from the nuclear envelope increases variability in 3D positioning of LADs within *Drosophila melanogaster* nuclei *Simon Bondarenko*[1,2], Igor Sharakhov[1,2] 1) Department of Entomology and the Fralin Life Sciences Institute, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061, USA; 2) Department of Genetics and Cell Biology, Tomsk State University, Tomsk, Russian Federation

The attachment of chromatin to the nuclear envelope correlates with gene repression, which may play an important regulatory role in the interphase nucleus. Lamins interact with the internal nuclear membrane (INM) and together with other proteins form nuclear lamina. Lamina-associated domains (LADs) of chromatin are shown to interact with the nuclear lamina, however the role of lamins in the 3D position of LADs inside the nucleus is not well understood. In this study, we developed LAD- and nonLAD-specific fluorescent oligo-probes for the chromosome 3 of *Drosophila melanogaster* and hybridized them with the wild type (wt) Canton S and Lam[A25] mutant nuclei. This mutant lamin lacks the hydrophobic CaaX box responsible for tethering the lamin Dm0 to the INM. The mutant Dm0 protein is not confined to the nuclear periphery but is distributed throughout the nuclear interior, colocalizing with chromosomes. We performed the confocal microscopy of highly-polytenized nuclei of salivary glands (SG) and low-polytenized nuclei of proventriculus (PV) labeled with LAD- and nonLAD-specific probes using Zeiss LSM 880 with Airyscan module. The radial distribution of DAPI intensity was measured by the radial profile plugin for Fiji software and the radial distribution of LADs and nonLADs was analyzed with the TANGO plugin for Fiji. The radial distribution of chromatin in Lam[A25] nuclei was significantly shifted toward the center of the nucleus in comparison with the wt, which suggests that the lamin Dm0 is necessary for the attachment of chromatin to the NE. Although, we did not detect a significant difference in the radial distribution of LADs and nonLADs between the mutants and wt (PCA analysis), the variability in LADs in the PV nuclei was 29% higher in Lam[A25] than in wt (significant, t-test, $p \leq 0.5$), whereas the variability in the radial position of LADs in SG was only 10.5% higher in Lam[A25] than in wt (significant, t-test, $p \leq 0.5$). At the same time, the variability of the radial position of nonLADs was not significantly different among all groups. The results suggest that the Dm0 protein plays an important role in the 3D position of LADs inside the nucleus.

560V ORC associates with the Nup107-160 subcomplex, coupling nucleoporins to replication initiation *Logan Richards*[1], Christopher Lord[1], Mary-Lauren Benton[2], John Capra[1,3], Jared Nordman[1] 1) Department of Biological Sciences, Vanderbilt University, Nashville, TN ; 2) Department of Computer Science, Baylor University, Waco, TX ; 3) Bakar Computational Health Sciences Institute and Department of Epidemiology and Biostatistics, UCSF, San Francisco, CA

The regulation of DNA replication is critical to ensure the accurate duplication of genetic information and maintain genome stability. Replication initiates at thousands of replication start sites throughout the genome. The origin recognition complex (ORC) binds throughout the genome to initiate DNA replication. In metazoans, however, it remains largely unknown how ORC is targeted to replication origins to facilitate helicase loading and replication initiation. We hypothesized that ORC's genomic binding was driven through protein-protein interactions. To address this, we performed immunoprecipitations coupled with mass spectrometry for ORC2, a subunit of ORC, in Drosophila embryonic extract. Surprisingly, we found that ORC2 associates with several subunits of the Nup107-160 subcomplex of the nuclear pore. We determined that this interaction is developmentally regulated, occurring most strongly in the first six hours of embryogenesis. Bioinformatic analysis revealed that, relative to all modENCODE factors, nucleoporins are the most enriched factors at ORC2 binding sites. Critically, ORC2 binding to chromatin is dependent on ELYS, a member of the Nup107-160 complex. Consistent with a function in ORC2 loading, knock down of ELYS delays S phase entry and impairs DNA synthesis. Our work reveals a new connection between ORC, replication initiation, and the nuclear pore. We propose that specific nucleoporins have a previously unrecognized function in metazoan replication initiation.

561V Hinfp is a guardian of the somatic genome by repressing transposable elements *Niraj Nirala*, Y Tony Ip University of Massachusetts Chan Medical School, Worcester, MA

Large portions of eukaryotic genomes contain repetitive sequences that include transposable elements, which can be mobilized to generate new integrations in the host genome, therefore affecting genome function, stability and evolution. Germ cells possess the Piwi-interacting RNA (piRNA) pathway to repress transposable elements to maintain genome stability across generations. Whereas, uncontrolled transposable element expression in somatic cells causes mutations that do not get passed on to future generations but may lead to pathological consequences. We have uncovered that loss of function of a single zygotic gene, *Hinfp*, which encodes a conserved zinc finger transcription factor, is sufficient to cause de-repression of most transposable elements, resulting in a substantial DNA damage in somatic tissues. Deep

sequencing, mutant clonal analyses and cell type specific RNAi experiments reveal that the key cell-autonomous target of Hinfp in this process is the linker histone *Histone1*, which has important roles in heterochromatin chromatin formation and transposable element repression. Pharmacological inhibition of reverse transcriptase by a combination of 3TC and AZT resulted in rescue of DNA damage in gut enterocytes. Moreover, transgenic expression of Hinfp or Histone1, but not Histone4 of core nucleosome, was sufficient to rescue the defects in repressing transposable elements and host genes. We also demonstrated that down regulation of Hinfp caused neurological defects in aging flies and enhanced the Ras oncoprotein-induced lethality and tissue growth. Overall, our findings suggest that Hinfp acts as a pivotal physiological regulator of Histone1- dependent silencing of most transposable elements, as well as many Drosophila genes, and serves as a new venue for studying genome stability, cancer progression, neurodegeneration and aging.

562V Dual loss of HP1B and HP1C impacts chromatin structure *Sarah Sims*, Nicole Riddle University of Alabama at Birmingham

Heterochromatin Protein 1 (HP1) proteins are non-histone chromosomal proteins that are highly conserved in eukaryotes. HP1 proteins can form homo- and heterodimers, which bind to other chromatin elements such as histones, DNA, and a variety of protein partners. Due to their diverse nuclear functions and maintenance of chromatin states, HP1 proteins are essential for ensuring the safety and functions of the genome. 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 functions, 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-mutant fly strains lacking HP1B and HP1C. We find that the viability of these animals is strongly dependent on genetic background, with one background producing a homozygous viable, fertile, and healthy stock despite lacking two HP1 proteins, while another background allows less than 10% of animals to survive. Examination of polytene chromosomes from these *HP1b/HP1c* mutants suggests that their morphology is affected, while HP1a continues to localize to the centromeres and telomeres. Our data demonstrate that complete loss of HP1B and HP1C is survivable in *D. melanogaster* and demonstrate the importance of genetic background. Ongoing studies focus on how pairwise HP1 loss affects gene expression and high-resolution chromatin analysis through CUT&RUN. 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.

563V Repair of double-strand breaks in *Drosophila* polycomb bodies Marieke Wensveen, Aditya Dixit, *Aniek Janssen* University Medical Center Utrecht

Eukaryotic cells are continuously exposed to DNA damaging insults that can break or chemically modify their DNA. Double-strand breaks (DSBs) are particularly dangerous because their improper repair can directly lead to insertions, deletions, or major structural chromosomal rearrangements. DNA is packaged into a variety of chromatin domains, which each have specific molecular and biophysical properties that can influence the DSB response. Whereas euchromatin contains open, actively transcribed regions, heterochromatic regions mainly consist of compact, silent genomic regions. One major type of heterochromatin is facultative heterochromatin, which is essential to silence developmental genes throughout organismal development. Facultative heterochromatin is enriched for trimethylation of histone 3 lysine 27 (H3K27me3) as well as polycomb proteins, and accumulates in sub-nuclear foci, called polycomb bodies. Although the DNA repair response in euchromatic regions has been extensively studied, the repair response in polycomb-enriched chromatin remains largely unknown. We hypothesize that this heterochromatin subtype requires distinct chromatin- or DNA repair- responses to ensure safe DSB repair. To study the DSB response in polycomb chromatin, we here integrate a previously established inducible single DSB system (A. Janssen et al. G&D 2016) in *Drosophila* tissue in either euchromatin or polycomb chromatin. Using this system, we find that DSBs in polycomb chromatin employ the canonical repair pathways Homologous Recombination and Non-Homologous End-Joining. Moreover, chromatin analysis reveals local changes in the *f*-Het chromatin landscape specifically at the break site. We hypothesize that these DSB-induced chromatin changes are necessary to detect, access or process the DNA break. Together, our data indicate that DSBs in polycomb chromatin use canonical DSB repair pathways and require specific local chromatin changes for their faithful repair.

564V Ectopic heterochromatin triggered by insertion of repetitive DNA is temperature-sensitive *Melissa Sawyer*[1,2], Safiyo Aden[1,2], Zayid Dakane[1,2], Nathan Dupre[1,2], Jack Jurmu[1,2], Hunter Lindsay[1,2], Heidi J.J. Pipkin[1,2], Anthony Ruiz [1,2], Jessica Xiong [1,2], Luke Ziegler[1,2], Andrew M. Arsham [1] 1) Bemidji State University, Bemidji, MN; 2) North Hennepin Community College, Brooklyn Park, MN

Heterochromatin is a key genomic defense against invasive genetic elements, mitigating damage by inhibiting transposition, silencing gene expression, and reducing recombination at insertion sites. How genomes recognize and silence novel threats prior to establishing sequence-specific adaptive defenses like piRNAs is poorly understood. To investigate genome defense against novel repetitive DNA we carried out a transposition mutagenesis screen, mobilizing a

reporter construct expressing the white gene adjacent to a 256-copy tandem array of the lac operator sequence from *E. coli*. We isolated insertional mutants with variegated eye color suggesting silencing by heterochromatin. Surprisingly, the observed variegation is exceptionally sensitive to rearing temperature despite different genomic locations and chromatin contexts. Contrary to classical studies of position effect variegation at constitutive heterochromatin, ectopic heterochromatin variegation was suppressed when flies were reared at 18°C and enhanced at 25°C. Suppression of variegation at lower growth temperatures may provide clues to the genetic and biochemical mechanisms of genome defenses against novel invasive DNA.

565V Nurf301 and Su(Hw) coregulate gene expression and nuclear organization through the recruitment of CP190 *shue chen*[1], Leah Rosin[1], Gianluca Pegoraro[3], Nellie Moshkovich[2], Patrick Murphy[2], guoyun yu[2], Elissa Lei[1] 1) Laboratory of Biochemistry and Genetics, National Institute of Diabetes and Digestive and Kidney Diseases,NIH, Bethesda,MD; 2) Laboratory of Cellular and Developmental Biology, National Institute of Diabetes and Digestive and Kidney Diseases,NIH, Bethesda,MD; 3) Center for Cancer Research, National Cancer Institute, NIH, Bethesda,MD

Chromatin insulators are DNA-protein complexes that promote chromatin 3D organization by mediating interactions between distant genomic sites. Insulators can prevent the spread of repressive chromatin and block communication between enhancers and promoters to regulate gene expression. Altering insulator function can lead to defects in cellular differentiation and organismal development. In *Drosophila*, the well-studied *gypsy* insulator consists of three core proteins: Su(Hw), Mod(mdg4)67.2, and CP190. Multimerization of insulator proteins forms insulator bodies, of which normal localization is correlated with proper insulator function. To identify factors required for insulator body formation, we used a cell line expressing a functional Mod(mdg4)67.2-GFP fusion protein and performed a high-throughput visual RNAi screen. This strategy identified Nurf301, a nucleosome remodeling factor, as a potential novel regulator of *gypsy* insulator body formation.
Previous work showed that nucleosomes are shifted at Su(Hw) sites in Nurf301 null hemocytes, but it remained unclear how Nurf301 mechanistically affects *gypsy* insulator function. First, we found that Nurf301 promotes *gypsy*-dependent insulator barrier activity in a ubiquitous manner using an *in vivo* luciferase assay. Co-IP and IP-mass spec results indicated that Nurf301 physically interacts with CP190, Su(Hw), and Mod(mdg4)67.2. Furthermore, Nurf301 co-localizes with insulator proteins, and depletion of Nurf301 extensively alters the distribution of *gypsy* insulator proteins on chromatin. ChIP-seq and NeuRNA-seq profiles in cells lacking Nurf301 and Su(Hw) revealed that Nurf301 and Su(Hw) mutually affect the binding of one another on chromatin and regulate expression of a similar set of genes. Correlation between altered nucleosome positioning and reduced Su(Hw) binding in Nurf301 null cells suggested a functional relationship. Analysis of chromatin accessibility using ATAC-seq supported the hypothesis that Su(Hw) serves as a docking site for recruitment of Nurf301, and subsequently, CP190. Finally, DNA Oligopaint FISH demonstrated that nuclear compaction of *gypsy* binding sites is specifically reduced with concomitant loss of CP190 binding after depletion of Nurf301. By providing evidence of mutual functional interaction between Nurf301 and Su(Hw), our data provide new insights into how a nucleosome remodeling factor interacts with insulator proteins to regulate the nuclear organization and gene regulation.

566V Essential role of Cp190 in physical and regulatory boundary formation Anjali Kaushal[1], Julien Dorier[1], Bihan Wang[1], Giriram Mohana[1], Pascal Cousin[1], Nicolas Guex[1], Erez Lieberman Aiden[2], *Maria Crisitna Gambetta*[1] 1) University of Lausanne, Lausanne, Switzerland; 2) Baylor College of Medicine, Houston, Texas

Animal genomes fold into contact domains defined by enhanced internal contact frequencies with debated functions in establishing independent gene regulatory domains. A large fraction of mammalian contact domain boundaries form by stalling of chromosomal loop-extruding cohesin by CTCF, but most *Drosophila* boundaries form CTCF-independently. However, how CTCF-independent boundaries form and impact organismal development remains largely unexplored. Here, we assess genome folding and transcriptional regulation defects in fly embryos completely lacking the ubiquitous boundary associated factor Cp190. We find that sequence-specific DNA-binding proteins like CTCF and Su(Hw) directly interact with Cp190-containing core complexes and recruit Cp190 to form most promoter-distal boundaries. Cp190 is essential for early development and prevents regulatory crosstalk between gene loci that pattern the embryo. Cp190 is thus currently the major player in fly boundary formation and function, revealing that diverse mechanisms evolved to partition genomes into independent regulatory domains.

567V Analysis of nuclear organization and dosage compensation in *Bombyx mori* by Oligopaint FISH reveals divergent 3D architecture between moths and flies Leah Rosin, Chen Dahong, Chen Yang, *Elissa Lei* NIH

Interphase genomes are organized into an intricate three-dimensional (3D) structure that facilitates accurate gene expression and maintains genome stability. This organization is largely conserved, yet how karyotype evolution influences 3D genome organization remains largely unexplored. Here, we use DNA Oligopaints to visualize autosomes and the Z sex chromosome (chZ) in *B. mori* (N=28) and compare our findings to *D. melanogaster* (N=4). We show that *B. mori* nuclei are highly compact, with nuclear volumes similar to *D. melanogaster* despite the *B. mori* genome being over three times larger. This discrepancy in nuclear size cannot be explained by differences in inter-chromosomal interactions or chromosome territory (CT) formation: both species harbor spatially separated CTs with minimal intermixing.

Furthermore, CTs in both species are non-randomly organized, with gene-poor chromosomes being most peripheral. However, homologous chromosome copies share a single CT in *D. melanogaster* due to somatic homolog pairing, which does not occur in *B. mori*. Moreover, we find significant differences in intra-chromosomal interactions. Unlike loose chromosome folding observed in *D. melanogaster*, *B. mori* chromosomes are tightly folded within CTs, with nearly all tested chromosomes showing interactions between both telomere domains and the center of the chromosome.

Our first-time visual analysis of individual chromosomes further allowed us to address the outstanding question of how dosage compensation (DC) is achieved in *B. mori*, a ZW species. In *B. mori* males, both chZs are similar in size and shape and are more compact than autosomes or the female chZ, suggesting that both male chZs are partially and equally downregulated during DC. This mechanism contrasts with DC in *D. melanogaster*, where the single male chX is upregulated. Thus, *B. mori* DC is instead more similar to DC in the nematode *C. elegans*. Using both FISH and ATAC-seq, we find that the female chZ repositions toward the nuclear center and the chromatin becomes more open concomitant with increased Z-linked gene expression at the onset of DC. Together, these studies represent the first non-sequencing-based support for Ohno's hypothesis for the evolution of dosage compensation. We uncover significant differences in chromosome folding between *D. melanogaster* and *B. mori* and intriguing similarities between DC in *B. mori* and *C. elegans*, despite these lineages harboring evolutionarily distinct sex chromosomes (ZW/XY). We propose a model where holocentricity may be more influential for interphase genome organization and DC than evolutionary relatedness.

568V Details of transgene construction determine effective siRNA production *Sudeshna Biswas*, Victoria Meller Wayne State University

Flies correct for imbalance of X chromosome dosage between the sexes by increasing X-linked gene expression in males. The Male Specific Lethal (MSL) complex, composed of proteins and roX RNA, localizes to the male X and participates in gene up-regulation. **How the X is identified remains unclear**. Loss of roX RNA causes mislocalization of MSL proteins and male lethality. Small interfering RNAs (siRNA) and satellite repeats enriched on the X contribute to X recognition. Our studies showed that ectopic expression of siRNA from 1.688^{3F} (cytological position 3F) partially rescues roX1 roX2 males and localization of the MSL complex, but siRNA from other repeats with similar sequence, including 1.688^{1A}, did not. We hypothesize that details of transgene construction could determine function. To test this, I engineered an siRNA-producing transgene with 1.688^{1A} sequence but with size, phasing and orientation identical to the biologically active 1.688^{3F} transgene. In accord with my hypothesis, the new 1.688^{1A} transgene rescued roX1 roX2 males to the same level as a 1.688^{3F} transgene. This suggests that details of transgene construction play a crucial role in production or processing of double stranded RNA. In the future, I will take advantage of siRNA-producing transgenes that differ in their ability to promote X recognition to determine if epigenetic marks at 1.688^{X} repeats are enhanced only when transgenes that promote X recognition are present. My studies will serve to disentangle the role of siRNA and chromatin modification in dosage compensation.

569V Aid from repeat-binding and architectural maintenance proteins important in *D. melanogaster* dosage compensation *Maggie Sneideman*, Victoria Meller Wayne State University

Drosophila melanogaster males carry one X and one Y chromosome, but females have two X chromosomes. To equalize the amount of expression of the X-linked genes between the sexes, males increase the expression of X-linked genes approximately two-fold. This is mediated by the Male-Specific Lethal (MSL) complex, which modifies chromatin to elevate expression. The MSL complex first binds at Chromatin Entry Sites (CES) on the X, and then spreads into nearby active genes. CES contain a short motif that is bound by the adapter protein CLAMP. CLAMP is necessary to attract the MSL complex to the CES. However, these motifs are also found on the autosomes. These autosomal motifs also bind CLAMP, but fail to recruit the MSL complex. 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 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. We selected known satellite DNA binding proteins, proteins with X-specific or male-specific effects, proteins with AT hook motifs, and heterochromatin factors. I will use knock down lines of my candidate genes to test for a male specific phenotype. I will mate these lines to a dosage compensation compromised line to determine if it genetically interacts with the CES. To determine if these proteins localize to the 1.688X repeats, ChIP for several candidates suspected to bind in this repeat region will be performed. Lastly, I will use Reem Makki's dual luciferase reporter assay to identify candidate genes necessary for recruitment of compensation by the 1.688^{X} repeats or CES. Thus far, ISWI, D1, and SAF-A appear to have a genetic interaction with the CES.

570V Investigating the function of Stonewall in the maintenance of *Drosophila* female germline stem cells *Ankita Chavan*[1,2,3], Madhav Jagannathan[1,2,3] 1) Institute of Biochemistry, ETH Zurich, Zurich, Switzerland; 2) Department of Biology, ETH Zurich, Zurich, Switzerland; 3) Life Science Zurich Graduate School, Zurich, Switzerland

The balance between stem cell self-renewal and differentiation is essential to maintain tissue homeostasis. Previous

studies have highlighted that the heterochromatin-associated protein, Stonewall (Stwl), is important for the maintenance of germline stem cells (GSC) in the *Drosophila* ovary; Stwl loss-of-function results in agametic ovaries while its overexpression results in an increase of undifferentiated cells. However, the mechanism by which Stwl maintains the GSC fate in the female germline remains incompletely understood. We hypothesized that Stwl regulates the balance between self-renewal and differentiation by modulating gene expression in GSCs. To test this, we measured gene expression changes upon overexpression of Stwl in a GSC-enriched population. Our data showed that Stwl overexpression was associated with the downregulation of genes encoding components of extracellular matrix, germline-enriched ribosomal genes as well as germline-associated cytoskeletal genes. Strikingly, we also observe that germline-specific knockdown of Stwl is associated with a specific loss of nuclear lamina in GSCs. Interestingly, tethering genomic loci to the repressive environment of the nuclear lamina has been shown to regulate cell-type-specific gene expression. As such, loss of the nuclear lamina may allow improper gene expression in Stwl-depleted GSCs, leading to their loss. Together, we propose that Stwl tethers differentiation genes to the nuclear lamina in GSCs and orchestrates a gene expression program that promotes self-renewal.

571V Investigating the consequences of histone overexpression in *Drosophila* *Risa Takenaka*[1,2], Harmit Malik [1,3] 1) Division of Basic Sciences, Fred Hutchinson Cancer Research Center, Seattle, WA; 2) Graduate Program in Molecular and Cellular Biology, University of Washington, Seattle, WA; 3) Howard Hughes Medical Institute, Fred Hutchinson Cancer Research Center, Seattle, WA

Eukaryotic genomes are packaged into chromatin, which is composed of histone-containing nucleosomes. 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. In *Drosophila*, the *abnormal oocyte* (*abo*) gene encodes a repressor of core histones. *abo* is a maternal-effect lethal gene first isolated by Larry Sandler. *abo* homozygous-mutant females produce fewer offspring relative to their heterozygous sisters. This defect can be attributed to an overproduction of histones, which disrupt the maternal-to-zygotic transition during embryogenesis. Despite *abo'*s important function in histone regulation, *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 have generated a precise, CRISPR/Cas9-mediated knockout of *abo* flies to accurately interpret the *in-vivo* consequences of histone overexpression. Using this *abo*-knockout fly, I will measure transcriptomic consequences of histone overexpression to potentially explain why different tissues and developmental stages might vary in their responses to histone overexpression.

572A Spargel/dPGC-1 is required in eggshell patterning and proper cytoskeleton organization during oogenesis and embryogenesis *Mohammed Shah Jalal*, Sabarish Nagarajan , Atanu Duttaroy Howard University, Washington, DC

PGC-1 (Peroxisome proliferator-activated receptor-gamma coactivator-1) is a robust activator of mitochondrial biogenesis in the liver and skeletal muscles. On the other hand, Drosophila PGC-1, Spargel (Srl), is predominantly expressed in the Drosophila ovary, and germline-specific knockdown of *srl* arrests late-stage egg chamber development, resulting in arrested oogenesis and female sterility. Unlike srl RNAi, *srl* hypomorphic mutants (srl^{1}/srl^{1}) have a marked decrease in growth rate, body size, life span, and fecundity. A CRISPR-mediated *srl* deletion (srl^{del}) is an amorphic allele that is embryonic lethal. But a trans-heterozygous mutant mother (srl^{del}/srl^{1}) is fertile and used to investigate further the effects of low dosage of Spargel on oogenesis. We determined the maternal requirement of Spargel during embryogenesis because trans-heterozygous (srl^{del}/srl^{1}) females produced deformed embryos, most of which remained unhatched. A range of embryonic deformities was noticed in eggs produced by srl^{del}/srl^{1} mother, which includes ventralized eggshells with phenotypes including single dorsal appendage (DA), 2 short DAs, or 2 DAs close together compared to the control. Broad (Br), a zinc-finger transcription factor that specifies the dorsal appendages forming cells, was not repatterned to clear the dorsal-anterior region in 10 of the 12 stages 10 egg-chambers investigated in srl^{del}/srl^{1} ovaries. Roughly, a third of the post-vitellogenic oocytes are found with Gurken mislocalization. Interestingly, more than 90% of the srl^{del}/srl^{1} egg chambers above stage 9 were found to have actin cytoskeletal defects, which might contribute towards dumping defects leading to small eggs. Follicle cell-specific knockdown of *srl* has minimum effect on dorsal appendage formation, implying that germ cell Spargel may regulate eggshell patterning via signaling interaction with follicle cells. We also found gaps in the distribution of the syncytial nuclei (nuclear fallout) in a majority of the embryos from srl^{del}/srl^{1} mother, which also show cytoskeletal defects in pseudocleavage furrow formation. Together, our findings suggest that maternal spargel is required for eggshell patterning and cytoskeleton organization during oogenesis and early embryonic development.

573B Identification and characterization of novel genes in *Drosophila's* retinal development utilizing a transcriptomics approach *Sequioa Smith*, Mardelle Atkins Sam Houston State University

All cells in a multicellular eukaryotic organism contain a complete genome. However, these organisms possess different cell types with diverse morphologies and functions; thus, highlighting the importance of the regulation of gene expression. Transcriptome data is essential for understanding differential gene expression during development. An

increasing stream of larger and more complex datasets has poured into public databases in the last decade. The storage and maintenance of this shared data are federally funded. Our research seeks to repurpose existing datasets to increase their public value while generating discoveries. Traditionally, genetic screens and isolation of spontaneous mutants have been used to identify factors that affect developmental processes. Mutant isolation and identifications are labor-intensive and time-consuming. We established a rapid data analysis strategy to identify candidate genes required for eye development, beginning with publicly available transcriptomes. Our transcriptome data analysis process produced a candidate list of 47 genes. I will present our strategy for candidate gene identification and initial characterization of several candidate genes.

574C Structure-function analysis of Defective proventriculus (Dve) in *Drosophila melanogaster* eye development *Anuradha Chimata*[1], Madhuri Kango-Singh[1,2,3,4], Amit Singh[1,2,3,4,5] 1) Department of Biology, University of Dayton, Dayton, OH; 2) Premedical Program, University of Dayton, Dayton, OH; 3) Center for Tissue Regeneration & Engineering (TREND), University of Dayton, Dayton, OH; 4) Integrative Science and Engineering (ISE), University of Dayton, Dayton, OH; 5) Center for Genomic Advocacy (TCGA), Indiana State University, Terre Haute, IN

Organ development is a highly regulated process to transform a monolayer organ primordium into a fully developed organ. Formation of morphogen gradient acts as critical cue to determine cell fate in developing tissues. In *Drosophila melanogaster* (Fruit fly), *wg* morphogen acts as a negative regulator of eye development and *wg* gradient determines the eye vs. head fate. Previously, we have identified *defective proventriculus (dve,* an ortholog of SATB1*)* as a novel dorsal patterning gene that regulates transcription of *wingless (wg)* morphogen. Axial patterning is required to establish Antero-posterior (AP), Dorso-Ventral (DV), and Proximo-Distal (PD) axes. Of the three axes, DV axis is the first lineage restriction event during eye development and any deviation results in developmental birth defects. In the dorsal gene hierarchy, *dve* acts downstream of GATA-1 transcription factor *pannier (pnr)* and upstream of *wg*. Loss-of-function of *dve* results in dorsal eye enlargement and increase in *wg* while gain-of-function results in eye suppression and reduced *wg* expression. In humans, *SATB1* functions as a transcriptional regulator and chromatin organizer and requires tetramerization by the ULD domain. Furthermore, SATB1, the human ortholog of *dve* is highly upregulated in cancers. Here we have used the *Drosophila* eye model to understand the role of different Dve protein domains in regulating *wg* during eye development. We hypothesized that different domains of Dve protein might be critical for regulating downstream target, *wg*. Understanding this can increase our knowledge of which domains might be pathogenic in human development or disease. We performed structure-function analysis of Dve protein to elucidate the role of various domains in regulating *wg* and eye development. We have developed several transgenic lines, which will allow us to induce expression of the specific domains of Dve protein and assay their effect in the eye. Dve has a ULD domain for tetramerization, HOX domains for DNA binding and PPP4R2 domain for H2AFX dephosphorylation. Here we present our results on ectopic induction of these domains and their effect on eye phenotype and *wg* expression in the developing eye. We demonstrate the requirement of tetramerization by ULD domain for Hox domain mediated eye suppression function of Dve and *wg* regulation during eye development.

575A Hh signaling coordinates stereotyped and stochastic patterns in the *Drosophila* eye *Alison Ordway*, Caitlin Anderson, Lukas Voortman, Elizabeth Urban, Robert Johnston Johns Hopkins University

Development of an organism requires both stereotyped and stochastic patterning. Stereotyped patterning robustly generates nearly identical structures across individuals. In contrast, stochastic cell fate specification produces randomized patterns that are unique to each individual. Stochastic fate decisions are required for the development of many sensory organs, including visual and olfactory systems. Despite their importance, how the molecular mechanisms controlling stereotyped and random patterns intersect within the same tissue has not been addressed. This project aims to determine how gene regulatory mechanisms are tuned to generate both highly regular and stochastic patterns within the same tissue using the *Drosophila* eye as a model.

The *Drosophila* eye is composed of ~800 ommatidia in a near perfect array. Each ommatidium comprises eight photoreceptors (R1-8) which develop in a predictable fashion. As photoreceptors are differentiating during larval eye development, a wave of morphogenesis driven by Hedgehog (Hh) signaling promotes the highly reproducible structure of the eye.

Underlying the uniform morphology of the fly eye is a random pattern of photoreceptor subtypes. Two R7 photoreceptor subtypes are defined by expression of light-detecting Rhodopsin proteins. Random patterning of these two R7 subtypes is controlled by stochastic ON/OFF expression of the transcription factor, Spineless (Ss). Ss^{ON} R7s express Rhodopsin 4 (Rh4), whereas Ss^{OFF} R7s express Rhodopsin 3 (Rh3). *ss* is regulated by an interplay of transcription and chromatin regulation during larval eye development.

I found that Hh signaling plays a second role in eye development to regulate stochastic patterning. *hh* mutants display a reduction in the percentage of Ss^{ON} R7s. Cubitus Interruptus (Ci), an effector of Hh signaling, binds at 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.

These results indicate that Hh signaling is finely tuned to drive both stereotyped eye morphology and generate stochastic R7 subtype patterning during development. My ongoing work aims to determine how Hh regulates transcription and chromatin compaction at the *ss* locus. I am also working to determine how Ci and Klu compete for binding and regulation of stochastic *ss* expression. My studies will describe how regulation of stochastic and stereotyped patterning is coordinated in a single developing tissue.

576B *Decapentaplgic* Regulates the Boundary Expression of *Midline* and *Groucho* in the Developing Eye Imaginal Disc of *Drosophila* *Alani Perkin*, Parag Bhatt, Britney Roberts, Sandra Leal Harris-Stowe State University

The development of an organism relies on interactions between genes within specific signaling networks across diverse tissues. The regulatory genes of these networks are responsible for programming the proliferation, cell fate specification, and differentiation of unique cell populations that create a tissue pattern central to organogenesis. A well-characterized family of developmental regulatory genes is represented by the T-box (Tbx) gene family that encodes transcription factor (TF) proteins sharing an evolutionarily conserved T-box DNA-binding domain. The Drosophila melanogaster T-box TF, *midline* (*mid*), has been shown to play an important role in cell fate determination and patterning within developing tissues such as the wing and leg imaginal discs as well as the embryonic nervous system. The Drosophila Transcription Growth Factor-beta (TGF-β) homolog *decapentaplegic* (*dpp*) is expressed within the morphogenetic furrow (MF) of third-instar larval eye imaginal discs which is a region critical for cell fate specification. Previous studies have uncovered a regulatory relationship between *mid* and *dpp* in cell fate specification and patterning. We hypothesize that *mid* and *dpp* execute a long-range cell signaling mechanism to regulate cell specification within the MF and patterning anterior of the MF. Using the UAS-Gal4 binary expression system, we found that *Dpp* maintains the proper boundaries of *mid* and Groucho (Gro) expression within the eye imaginal disc. We are now investigating whether the Notch-Delta signaling pathway plays a role in modulating *dpp* and *mid* activity to pattern the eye disc.

577C Insights into the evolution and development of stochastic *Drosophila* retinal patterning through cross-species comparison with yellow-fever mosquito, *Aedes aegypti* *Zachary Goldberg*, Julia Ainsworth, Yunchong Zhao, Crystal Diei, Michael Perry University of California San Diego

How do adaptations arise within the constraints of existing genetic programs? The vast diversity of insect eyes and downstream neural circuits present an ideal system for studying how evolution modifies developmental programs to create morphological and neural novelty. Because of the interconnectedness and physiological precision necessary for vision, any modification must be accommodated at multiple levels, including visual processing circuits in the brain. Insect eyes are made up of repeating “simple eyes” called ommatidia, and contain a surprising diversity of arrangements and modifications that provide specialized functions. In the Diptera (true flies), each ommatidium contains eight photoreceptors (PRs): six used for motion vision and two used to make color comparisons. Different subtypes of ommatidia have color vision PRs which express different combinations of Rhodopsins sensitive to different wavelengths of light. In Drosophila, stochastic distributions of PR subtypes ensure that different color detectors cover the entire visual field. In contrast, Aedes aegypti mosquito retinas sacrifice stochastic patterning to instead place a subset of color-sensitive Rhodopsins in a local, regionalized ventral stripe of unknown function. What genetic regulatory changes are needed to repattern the eye? How do downstream neural circuits adapt to changes in upstream retinal patterning? What constraint exists on evolution of a system as complex as vision? By leveraging the vast array of tools and knowledge in the stochastic Drosophila retina with the non-stochastic patterning of the *Aedes* retina, we seek to understand how insects have evolved to pattern their retinas at the level of gene regulation. Previous work has shown that the transcription factor Spineless (Ss) controls stochastic PR patterning in Drosophila. We are investigating the role of Ss in the *Aedes* retina to understand if gene regulatory changes at the Ss locus alone are sufficient to repattern the eye and rewire the brain. Studying this novel neural feature and how the rearranged receptors in the eye connect to the proper circuits in the brain will greatly enhance our understanding of how complex interconnected systems evolve.

578A The Goldilocks effect: proper dosage of PAX6 levels is required for proper retinal differentiation and patterning in Drosophila. *Claude Jean-Guillaume*, Justin Kumar Indiana University

The transcription factor PAX6 is the master regulator of eye development in all seeing animals. In *Drosophila melanogaster*, its orthologs Eyeless (Ey) and Twin of Eyeless (Toy) sit atop the retinal determination network (Rdn), which represents a group of around fourteen transcription factors and co-factors responsible for retinal specification and patterning. Many of PAX6’roles were first elucidated in *Drosophila* through studies of ey, and it’s been believed that the specific loss of *ey* would result in the collapse of the Rdn and the loss of eye development; while the early embryonic loss of *toy* results in a headless fly (both Ey and Toy are required for overall growth of the eye-antennal imaginal disc), *toy* knockdown during larval development does not affect the compound eye. However, eye development can proceed in a complete loss-of-function *ey* mutant background. Our big question is to understand how the fly’s PAX6

molecules regulate eye development. More specifically, we seek to demonstrate how these molecules cooperate to ensure proper expression of the general morphogen *decapentaplegic* which is required for proper patterning of the eye. Moreover, we wish to show how a proper balance between the expression levels of *ey* and *toy* is required for proper development of the two fly's visual systems, the ocelli and compound eyes, and dorsal-ventral boundary establishment within the compound eye. Using a set of genetic and molecular tools, we have shown that eye development can proceed in the absence of Ey due to Toy, which binds to and activates Ey targets. We have also shown that Ey plays a previously unknown role early in establishing the anterior dorsal head vertex field, which is the region that later gives rise to the ocelli. Early retraction of Ey from that region is required for proper ocellar and dorsal head vertex development. Finally, we have shown that loss of Ey can lead to a mis-regulation of dorsal-ventral patterning genes in the compound eye.

579B The timing of cell fate decisions is critical for initiating pattern formation in the Drosophila eye *Justin Kumar*, Bonnie Weasner Indiana University

Patterning of the compound eye begins at the beginning of the third larval instar when a wave of morphogenesis initiates from the posterior margin of the eye-antennal disc and sweeps across the eye primordium. The leading edge of this differentiating wave is visualized by a groove in the epithelium called the morphogenetic furrow. A number of signaling cascades including the Hedgehog, Decapentaplegic, JAK/STAT, EGF Receptor, and Notch pathways are required for the initiation of the furrow. If these pathways are disrupted early in development, then the furrow is prevented from initiating from the posterior margin. As a result, the developing eye field fails to be properly patterned and adult flies lack the compound eyes. These phenotypes are reminiscent of *eyeless* (*ey*), *eyes absent* (*eya*), and *sine oculis* (*so*) loss-of-function mutants. These genes belong to the retinal determination (RD) gene regulatory network and are responsible for specifying the fate of the compound eye. Interestingly, expression of *hedgehog* and *decapentaplegic* are lost when the RD network is disrupted. Based on these observations, it has been proposed that the RD network is reiteratively used to first specify the fate of the compound eye and then later to initiate pattern formation. Here we describe an alternate mechanism that explains the role that the RD network plays in pattern formation. We have made the unexpected discovery that the RD network functions within the margin of the disc to control the timing of cell fate decisions. In normal development, cells of the margin which surround the compound eye are transformed into head epidermis during mid to late pupal development. This is well after the eye has been completely patterned. These cells then generate a gradient of Wingless (Wg) signaling that, in turn, establishes distinct cellular fates along the periphery of the retina. The result is a clear and smooth transition from ommatidia to bristle laden head epidermis. We show that in RD mutant discs this transformation occurs prematurely during larval development. This precocious change in cellular fate appears to be the underlying reason for the collapse of *hedgehog* and *decapentaplegic* expression and for the failure of the morphogenetic furrow to initiate from the posterior margin. We propose that a cardinal role for the RD network is to control the timing of cell and tissue fate decisions.

580C Elucidating the role of the *Drosophila melanogaster* TENT5 homolog in eye development *Abdulqater Al-nouman*, Jennifer Curtiss New Mexico State University

Eyeless is a paired-homeodomain transcription factor that sits at or near the top of a network of transcriptional factors that initiate eye development in *D. melanogaster.* Eyeless is conserved across metazoans, with the human ortholog being Pax6. Transcriptional networks such as this are required for cell fate determination in all tissues especially during development when a single egg cell differentiates into the astonishing variety of cells seen in an organism. Previous transcriptomic data and chromatin immunoprecipitation sequencing (ChIP-seq) analysis suggest that the *D. melanogaster* gene CG46385 is a direct transcriptional target of Eyeless. CG46385 is the *D. melanogaster* ortholog of human TENT5, which has been shown to function as a non-canonical poly-A polymerase (PAP). PAPs are involved in modification of mRNA by adding long strings of adenine bases to the 3` end, which can affect mRNA stability and translational efficiency. Because CG46385 is a direct transcriptional target of Eyeless, we hypothesize that it has a role in regulating gene expression during eye initiation by regulating mRNA stability. We are performing fluorescent in-situ hybridization on larval eye-antennal imaginal disks and embryos to determine at what stage of development and in what cells CG46385 may function. In addition, we are generating CRISPR-Cas9 mediated CG46385 mutants and plan to observe the eye phenotypes of these mutants to determine CG46385's role in eye development. Our studies will provide insight into the function of the human ortholog TENT5, which has been linked to congenital diseases including retinitis pigmentosa and osteogenesis imperfecta.

581A Extradenticle expression in the *Drosophila Melanogaster* eye regulates ectopic patterning on the ventral margin of the eye-antennal imaginal disc *Jasmine Warren*, Bonnie Weasner, Justin Kumar 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 in 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. I hypothesize that the loss of *exd* leads to the loss of the ventral repression pathway of the eye field. 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.

582B Heterodimerization-dependent secretion of BMP5/7 is required for wing patterning in *Drosophila* *Milena Bauer*[1], Gustavo Aguilar[1], Kristi Wharton[2], Shinya Matsuda[1], Markus Affolter[1] 1) University of Basel, Basel, Switzerland; 2) Brown University, Providence, USA

One of the key processes of embryonic development is the establishment of differentiation patterns. These, in turn, tightly depend on cell-cell communication, often mediated by secreted signalling molecules. Morphogens are a paradigmatic example: upon secretion from a localized source, morphogens disperse forming concentration gradients that will activate distinct gene targets at different concentration thresholds. Bone Morphogenetic Proteins (BMPs) act as indispensable morphogens in multitude of scenarios. Different BMPs are often expressed in the same tissue acting, potentially, in a combinatorial manner, forming both homo- and heterodimers. While the biochemical properties of the different ligand combinations have been extensively studied, little is known about the impact that they have on morphogen gradient formation.
In the *Drosophila* developing wing there are two BMP-type ligands, Decapentaplegic (Dpp) and Glass bottom boat (Gbb). It has been proposed that they can form both hetero and homodimers. Although is assumed that wing development mainly requires Dpp, Gbb has also been shown to be involved in patterning and growth, despite relatively weak phenotypes. Based on genetic analysis it has been suggested that Dpp and Gbb exhibit different effective signalling ranges. However, dissection of these process exceeds the power of current genetic tools. We have generated *ad hoc* CRISPR reagents and protein binder-based tools to visualize and manipulate the dispersal of Gbb and Dpp.

583C Evolutionarily young genes *flf1* and *flf2* are required for Wingless signaling in the wing development of *Drosophila* *Yusuke Kurihara*, Yuka Doi, Tomoko Nakamura, Yoko Keira, Hiroyuki Ishikawa Graduate School of Science, Chiba University, Japan

Conserved and ancient genes often play essential roles in various biological processes, whereas young genes, which exist in only one or a few species, had been considered to perform relatively minor functions. Recent studies in *Drosophila* have demonstrated that young genes play important roles in morphogenesis and behavioral decision. However, little is known about the involvement of young genes in the development of the *Drosophila* wing. Here we show two young genes *four-jointed localization factor (flf) 1* and *flf2* function redundantly to regulate Wingless (Wg) signaling in the *Drosophila* wing development.
In a previous study, we identified *flf1* as a modulator of the cellular localization of the Golgi kinase Four-jointed. Moreover, we identified *flf2*, a paralogous gene of *flf1*, by using nucleotide BLAST against the *Drosophila* reference genome. *flf1* and *flf2* are tandemly arrayed genes, and the amino acid sequences of their translation products are highly similar. For further analysis of functions of *flf1* and *flf2*, we produced mutations of these two genes by using CRISPR/Cas9 system. Single mutant flies that lack *flf1* or *flf2* developed and reproduced without an obvious phenotype. By contrast, the number of chemosensory bristles was decreased in the adult wings of *flf1 flf2* double mutant. An abnormal number of chemosensory bristles in the adult wings is found in *wg* mutants. Therefore, we decided to investigate relationship between *flf(s)* and Wg signaling. Wg puncta diffusing away from the dorso-ventral boundary cells was decreased in the wing imaginal discs of *flf1 flf2* double mutant. This result indicates that *flf1* and *flf2* are required for normal expression pattern of Wg in the wing imaginal discs. To test whether Flf(s) affect Wg diffusion in a dose dependence manner, we overexpressed *flf1* or *flf2* in the wing imaginal discs. Overexpression of Flf1 or Flf2 enhanced Wg diffusion in the wing imaginal discs. Our results suggest requirement of *flf1* and *flf2* for proper expression pattern and function of Wg in the *Drosophila* wing development.

584A Defining the Role of *CG11617* in the Transcriptional Control of Muscle Development in *Drosophila*

melanogaster *Elizabeth Trujillo*, Richard Cripps San Diego State University, San Diego, CA

The mammalian *Mohawk* transcription factor is known to be expressed in embryonic precursors of skeletal muscle and functions by regulating the transcription of slow-twitch myosin-heavy-chain isoform expression in fast-twitch muscle fibers, through *Sox6* repression, during muscle development. However, we do not fully understand its mechanistic role in invertebrate skeletal muscle development. By using the *Drosophila melanogaster Mohawk* ortholog, termed *CG11617*, we first analyzed CG11617 localization. Thoracic flight muscles reveal nuclear CG11617 localization whereas the tergal depressor of the trochanter muscles (TDT, or jump muscles) reveal nuclear and cytoplasmic localization between the myofibrils. Analysis of CG11617 knockdown animals revealed that these flies are lethal in the pharate adult stage. Upon examination, these mutant flies showed fibrillar disorganization of indirect flight muscles, the absence of jump muscles, and a significant decrease in imaginal wing disc myoblasts compared to the wild-type. Together, these findings may account for the overall skeletal muscle impairment and pupal lethality observed in the knockdowns. Furthermore, utilizing Gal4 driver lines containing fiber-specific enhancer-lacZ constructs, to follow muscle fiber fate, we observed a fibrillar muscle to tubular muscle identity switch in these mutant flies. Overall, these findings suggest that *CG11617* may skew skeletal muscle precursor differentiation towards one fiber-type versus another, and *CG11617* is a determinant of skeletal muscle fiber-type specification and differentiation.

585B Single-cell sequencing of *Drosophila* embryonic heart and muscle cells during differentiation and maturation *Georg Vogler*[1], Bill Hum[1], Marco Tamayo[1], Yoav Altman[2], Rolf Bodmer[1] 1) Sanford Burnham Prebys Medical Discovery Institute, Development, Aging and Regeneration, La Jolla, CA; 2) Sanford Burnham Prebys Medical Discovery Institute, NCI-designated Cancer Center, La Jolla, CA

The developing *Drosophila* heart consists of cardial cells that differentiate into different types of cardiomyocytes and pericardial cells. A large body of work has identified numerous genes and pathways involved in heart specification and differentiation, downstream of cardiac transcription factors, such as Tinman (NKX2-5) and Dorsocross1/2/3 (TBX5). The advent of single-cell RNA sequencing (scRNAseq) technology allowed us for the first time to describe the transcriptome of different cardiac cell types in the Drosophila model at high resolution. Here, we applied scRNAseq on sorted cells of late-stage *Drosophila* embryos expressing a cardiac GFP reporter. We find distinct expression profiles of cardioblasts as they mature to cardiomyocytes, as well as discretely clustering pericardial cells, including a set expressing Tinman that potentially assist in heart morphogenesis. In addition, we describe other cell types that were sequenced as by-catch due to low but distinct extracardiac expression of the GFP reporter. Our studies on wildtype cardioblasts will be the foundation for investigating developmental profiles in mutant backgrounds and for generating gene regulatory networks at single-cell resolution during cardiogenesis.

586C Discs Large is a novel regulator of the Enteroblast Mesenchymal-to-Epithelial Transition in the adult *Drosophila* midgut *Fionna Zhu*, Michael Murray, Georgia Malloy University of Melbourne

Maintenance of epithelial barrier function is essential for human health. The adult *Drosophila* midgut provides a powerful model system for understanding mechanisms of epithelial homeostasis. It consists of a monolayered epithelium of absorptive enterocytes (ECs) and secretory enteroendocrine cells, underpinned by stem cells and intermediate progenitor enteroblasts (EBs). When EBs differentiate into ECs, they undergo a Mesenchymal-Epithelial Transition (MET) and intercalate into the epithelium.

While much is known about the transcription factors and signalling pathways that regulate EB identity and differentiation, the mechanical aspects of MET are less well studied. To identify novel EB MET regulators we conducted an RNAi screen using the lineage tracing tool, ReDDM. We screened genes likely to be involved in that process and identified three components of septate junctions (SJs): Discs Large (dlg), Mesh and Snakeskin.

Dlg is a member of the MAGUK family of scaffolding proteins and is known to regulate SJ formation, cell polarity and proliferation. EB-specific knockdown of *dlg* using Su(H)GBE-GAL4, resulted in a multilayered epithelium, a more rounded EB morphology, defective SJs and premature expression of the EC-marker Pdm1. *dlg* knockdown also led to a shortened lifespan and increased mortality due to chemical damage by DSS, likely as a result of impaired barrier function.

MARCM clones deficient for *dlg*, recapitulated the multilayering and SJ protein mislocalisation RNAi phenotypes. Interestingly, the extent of multilayering appears more severe in the anterior midgut compared to the posterior. Patches of Mesh and Ssk were mislocalised to the apical membranes of clones and were complementary to enriched F-actin, suggesting disruption of microvilli structure. We also observed intracellular actin-rich vacuoles (likely autophagosomes) and apical membrane disruption, features previously associated with delamination and cell death upon Ecc15 infection, coordinated by the EGFR pathway. Isolated clones were occasionally found detached from the basement membrane, supporting this hypothesis. Gaps in the epithelium were also found nearby clones, with enrichment of SJ proteins at their boundaries.

We are currently examining whether these phenotypes are caused by disruption to key signalling pathways. Together, our results highlight the role of Dlg in midgut homeostasis and MET and the utility of the *Drosophila* model to expand our understanding of epithelial homeostasis.

587A A role for the *apterous* gene in adult survival of *Drosophila melanogaster* *Cindy Reinger*, Michèle Sickmann, Markus Affolter, Martin Müller Universitiy of Basel

The *apterous (ap)* gene codes for a transcription factor belonging to the LIM-homeodomain family. *ap* is best known for its role in the patterning of the adult wing where it determines the identity of cells in the dorsal compartment. We have previously shown that the expression of *ap* in the wing relies on two tissue-specific enhancers, apE and apDV. The phenotypic consequences of an apE deletion are viable and fertile flies devoid of wings, which can be maintained as a homozygous stock. True *ap* null alleles also produce wingless adults. However, and in contrast to ΔapE, such flies also show the precocious adult death syndrome. This condition had attracted some interest ~40-60 years ago from investigators like King, Butterworth and Wilson. They reported that the syndrome correlates with female sterility, abnormal adipose tissue and adult death within 72 to 96 hrs after eclosion. Among the three, the latter phenotype is the most obvious and striking: *ap* null flies readily hatch in good numbers and are active. But after about one day, they become lethargic and die within the next 72 hrs. A model for the comprehensive explanation of the syndrome and the connection between the individual disorders is still missing.
In the course of our studies on the *ap* locus, we have recently started to address the genetic and cellular basis of the precocious adult death syndrome. We will present an overview of our initial results.

588B Patterning and Morphogenesis of the Posterior Midgut *Daniel S. Alber*[1,2], Maria Avdeeva[3], Liu Yang[2], Shannon E. Keenan[1,2], Eric F. Wieschaus[2,4], Stanislav Y. Shvartsman[2,3,4] 1) Department of Chemical and Biological Engineering, Princeton University, Princeton, NJ, USA; 2) The Lewis-Sigler Institute for Integrative Genomics, Princeton University, Princeton, NJ, USA; 3) Center for Computational Biology, Flatiron Institute, Simons Foundation, New York, NY, USA; 4) Department of Molecular Biology, Princeton University, Princeton, NJ, USA

The organogenesis of the *Drosophila* posterior midgut (PMG) offers a genetic and morphological model system to study the fundamentals of tissue patterning and morphogenesis in a highly conserved and broadly relevant tissue. ERK-mediated signaling acts primarily through two key regulators, Tailless (Tll) and Huckebein (Hkb), to pattern the posterior region of the embryo prior to gastrulation. We use immunostaining to follow the expression patterns of 16 potential targets of these regulators, including overlapping domains of Brachyenteron (Byn), a highly conserved T-box transcription factor, and Wingless (Wg). We elaborate on the conventional model of specification to include effects of Tll and Hkb mutants on the expression patterns of these 16 targets and establish a hierarchy of expression. We reveal the dynamics of this signaling network via transcriptional live reporters created via CRISPR and live imaging using light-sheet microscopy. Using a novel computational method to fuse imaging datasets of the individual components of posterior patterning into a common multivariable trajectory, we show a low intrinsic dimensionality of posterior patterning. Additionally, we classify the morphological changes as the primordial mid- and hind-gut invaginates and involutes into distinct geometric transformation events and connect it to spatiotemporal expression patterns of key PMG-specifying markers. We focus on the morphological effects of Byn, which plays a key role in the cell shape changes and rearrangements in zebrafish and frog embryogenesis, by studying PMG morphogenesis in various mutant backgrounds. Our analysis supplements the existing understanding of epistatic relationships within the PMG-specifying gene regulatory network and describes PMG morphogenesis in the context of gene expression patterns.

589C Identifying split-GAL4 drivers for targeting and manipulating enteroendocrine cells in the Drosophila midgut *Jessica Holsopple*[1,2], Ellen Popodi[1,2], Kevin Cook[1,2] 1) Indiana University, Bloomington, IN; 2) Bloomington Drosophila Stock Center, Bloomington, IN

The Drosophila midgut is a valuable model tissue in many ways, one of which is its utility for characterization and manipulation of cells involved in intestinal endocrine signaling. As in other organisms, the Drosophila intestine contains a group of secretory cells called enteroendocrine cells. This unique group of cells releases peptide hormones to induce systemic effects that allow the whole organism to respond to changing conditions in the gut. Enteroendocrine cells are often divided into subcategories based on the profile of peptides a cell secretes. The diversity of these cells requires methods of identifying, targeting, and manipulating subpopulations of them to clearly understand the functions of discrete subgroups. The split-GAL4 system provides a promising avenue to address this issue and previous studies have identified many combinations of split-GAL4 drivers that target small groups of enteroendocrine cells. Our current work investigates select pairs of split-GAL4 drivers and characterizes the cells reported by these pairs using the expression profiles of peptide-encoding genes reported by lexA knock-in drivers known to target common, midgut-secreted peptides. Using this technique, we are able to characterize the subset(s) of enteroendocrine cells that a split-GAL4 pair reports. We also test various double reporters containing both UAS and lexAop components and analyze the strengths and weakness of these reporters when compared to each other. Overall, this work provides evaluation of lexA drivers for select peptides, detailed characterization of split-GAL4 driver pairs that allow for manipulation of known subpopulations

of enteroendocrine cells, and a framework for future, straightforward characterization of such pairs not characterized in this study.

590A Characterization of novel *Drosophila* Egf receptor signaling targets with roles in eggshell structure and morphology *Molly Yuschock*, Jessica White, Taylor Reiff, Lisa Kadlec Wilkes University

Drosophila epidermal growth factor receptor (Egfr) signaling plays a critical role in many aspects of development including oogenesis, embryogenesis, and proper development of wing and eye tissues. For example, during wing development, Egfr signaling helps specify vein tissues, and in the ovary Egfr signaling is known to establish the body axes during oogenesis. Microarray screens by our lab and others have identified potential downstream transcriptional targets of the Egf receptor using the *Drosophila* ovary as a model system. Our initial work compared gene expression in fly ovaries where the activity of the Egfr pathway was reduced (gurken mutant), wild-type (OreR), or constitutively active (CY2/λTop). We have employed a number of approaches to further investigate the expression, biological function, and mechanism of action of a subset of putative genes of interest, focusing primarily on genes of previously unknown function. A small-scale functional screen using available collections of UAS-RNAi transgenic flies and P-element insertion lines was used to investigate the possible functions of a group of these novel EGFR-responsive genes. A number of these genes were observed to play roles in normal eggshell structure and morphogenesis. Gene mutant/knockdown phenotypes include decreased chorionic integrity, shortened eggs, and various dorsal appendage malformations, as well as decreased fertility. We have used the CRISPR-Cas9 system to create mutations in some of these "morphogenesis genes." These mutants have so far recapitulated the previously observed phenotypes, and in at least one case resulted in the observation of an additional phenotype in our null mutant, not seen in the original P-element insertion. We are currently using these CRISPR mutants for further study and characterization of the genes.

591B Characterizing the Role of Doublesex in Creating Sexual Dimorphism in the Somatic Gonad *Natalie Murphy*, Ellen Baxter, Mark Van Doren Department of Biology, Johns Hopkins University, Baltimore, MD

The Doublesex (Dsx) and Mab-3 Related Transcription factor (DMRT) family proteins are paramount for sex determination in most animals, from planaria to flies, birds, mice, and humans. In *Drosophila*, the embryonic gonad is formed when a bipotent cluster of somatic gonadal precursors (SGPs) coalesces with the germ cells. Sex determination is regulated by X chromosome dosage, which activates an alternative splicing cascade that yields Dsx^F in females and Dsx^M in males. Both Dsx isoforms have the same DNA binding domain, but they 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 taking a multi-pronged approach to investigate the cell fate decisions that are regulated by Dsx, as well as the target genes it controls to make these decisions. First, we used an endogenously tagged GFP-Dsx to characterize Dsx expression in the somatic gonad. We found that throughout development, males express Dsx in the early somatic support cells (i.e., the hub and early cyst cells), as well as the terminal epithelium. Female Dsx expression is dynamic throughout development. By adulthood, Dsx is limited to the early somatic support cells (i.e., the cap cells and escort cells) – this is a similar pattern as seen in the male. However, at pupal and larval stages, Dsx expression is more promiscuous, and is seen in a wider range of somatic cell types.

This points towards an early "establishment" need for Dsx in some cell types, and a more limited need for "maintenance" as the gonad ages. We will use small *dsx* LOF clones to further investigate this hypothesis, which cells of the gonad require autonomous sex information, and which cells may be regulated through cell-cell signaling. Ultimately, we aim to elucidate the mechanisms of Dsx in regulating the sex determination of *Drosophila* gonad somatic stem cell development.

592C The role of the extracellular protease AdamTS-B and BMP signaling in wing vein formation *Olivia De Grace*, Afshan Ismat University of St. Thomas

The Drosophila wing is composed of longitudinal veins (L1-L6) and crossveins, the posterior crossvein (PCV) and anterior crossvein (ACV). AdamTS-B is one of three ADAMTS proteins expressed in Drosophila, specifically in the wing imaginal disc. It was previously shown that loss of *AdamTS-B* in the wing resulted in extra PCVs or deltas. Moreover, over-expression of this protease resulted in a complete absence of the PCV and distal end of the L5 longitudinal vein. The BMP pathway is known to play an essential role in wing vein formation, especially the PCV. The question we are asking is whether AdamTS-B functions through the BMP signaling pathway to inhibit wing vein formation. In order to explore this question, we are performing genetic interaction studies between *AdamTS-B* and components of the BMP signaling pathway to examine whether these two proteins function in the same signaling pathway or not. Using the GAL4/UAS system of over-expression, we are using a wing-specific GAL4 line (*MS1096*-GAL4) to over-express combinations of *AdamTS-B* and each of the following BMP signaling pathway components: *dpp, gbb,* and *tkv*. I predict that over-

expression of these BMP pathway components may counteract the defects seen in *AdamTS-B* over-expression and result in a more "normal" wing vein pattern. The anticipated impact of this research will be more knowledge of how this important BMP signaling pathway is regulated. We will also learn more about potential functions of the *AdamTS-B* gene in other tissues as it is also expressed in the embryonic trachea.

593A A single cell atlas of *Drosophila* embryonic epidermal and salivary gland cells highlights spatiotemporal gene expression during tube morphogenesis *Annabel May*, Katja Röper MRC LMB

Many internal organs are composed of epithelial tubes, the morphogenetic effectors responsible for controlling morphogenesis in these tubes and their order of action is yet to be comprehensively assessed. Within our tubulogenesis model, the *Drosophila* embryonic salivary gland, morphogenetic effectors have previously been identified either individually, as a result of mutant phenotypes and whole embryo microarrays or systematically, though gain of function screens. Currently there has been no attempt to assess the entire transcriptional landscape of the developing *Drosophila* melanogaster embryonic salivary gland.

Using a combinatorial method of strictly staged embryos, fluorophore driven embryo cell sorting and 10x chromium single cell sequencing we describe two new stage 11 embryonic single cell sequencing data sets. A comprehensive cell lineage of the embryonic salivary gland, central nervous system and muscular cells driven by the transcription factor *forkhead* covering developmental time points of salivary gland specification, invagination and migration and a second dataset at an identical time point isolating epithelial cells expressing the *Drosophila* homologue of Beta-Catenin, *Armadillo*.

Here we present both data sets as a spatiotemporal specific bioinformatic tool to identify and investigating genes involved in salivary gland development. Following bioinformatic labelling of the two cell populations we employed differential expression analysis. This technique alone yielded well-known salivary gland markers and a number of novel genes not previously implicated in salivary gland development. These genes include salivary gland specific and early expressed transcription factors, highly up-regulated genes within the developed salivary gland, genes specifically excluded from the salivary gland compared to surrounding epithelia, and a number of as-yet uncategorised genes. Further investigation into the *forkhead* driven cell population using pseudotime and marker based identification provided a useful tool for prediction of temporal based expression across salivary gland development. This technique again successfully identified both well-known genes and genes previously not described to be temporally expressed within the salivary gland. This specific dataset has lead to the identification of novel gene expression patterning within the early salivary gland, identification of temporally controlled expression of adhesion molecules in time with morphogenetic movement of the tissue and highlighted upregulation of secretory machinery far earlier than previously reported.

594B Using NaNuTrap method to provide insight into synchronized remodeling of adjacent tissues ectoderm and mesoderm at gastrulation *Zsuzsa Akos*, Angelike Stathopoulos California Institute of Technology

Early large scale tissue remodeling often happens concomitantly in neighboring tissues in embryos. In *Drosophila melanogaster*, the fast phase of germ band extension (GBE) that drives stretching in the ectoderm happens at the same time as cell division in the neighboring mesoderm. The concurrence of these processes suggests that this synchronization relates to (i) a molecular mechanism that induces division at the same time as GBE happens or, alternatively, (ii) cell division in mesoderm is a response to tension created by the ectoderm. The latter mechanism would ensure coordination between these processes and help release the tension when it reaches a level too high to tolerate. We recently developed a new method, NaNuTrap that utilizes maternally deposited fluorescent proteins (FPs) and zygotically expressed nanobodies to label tissues early in development, which previously was challenging in *Drosophila* due to its fast development compared to the maturation time of the FPs. We use NaNuTrap to label the mesoderm and separate it from the adjacent ectoderm in order to track cell movement and division in these tissues. In this way, we can investigate whether the more detailed spatial pattern of cell division in the mesoderm also follows the stretching pattern of the adjacent ectoderm tissue in wildtype embryos. We also seek to determine whether cell division in the mesoderm would be affected if tension created by the ectoderm is altered. In summary, we use the NaNuTrap method to investigate if these two processes, stretching of ectoderm and division of mesoderm cells, are coordinated through physical attachment or by synchronized molecular pathways that induce them at the same time.

595C The small GTPase Rap1 promotes polar cell survival and morphogenesis to form the migratory border cell cluster *Luke Messer*, Jocelyn McDonald Kansas State University

So-called 'organizer cells' help other cells develop through directing cell fate specification, stem cell maintenance, and even cell migration. Often these various organizer cell functions are due to production of secreted, diffusible signals that initiate specific responses in neighboring cells. One such organizer is the polar cells that form at the anterior and posterior poles of egg chambers, the functional units of the developing ovary. Polar cells are initially produced in excess. Regulated apoptosis eliminates these 'supernumerary' polar cells to produce the 'mature' polar cell pairs. Later in

oogenesis, the anterior polar cell pair secretes the cytokine Unpaired to activate JAK/STAT signaling in adjacent follicle cells, which become migratory border cells. If excess polar cells survive, too many border cells are recruited, whereas if one or both mature polar cells die, too few border cells form, either of which inhibits successful migration. Thus, survival and maintenance of the mature polar cells must be tightly regulated so only two polar cells survive, yet the mechanisms regulating this are unclear. Here we show that the small GTPase Rap1 controls border cell cluster size by promoting survival of the mature polar cells. We found that egg chambers deficient for Rap1 form border cell clusters with fewer cells than normal. At least 20% of border cell clusters were missing at least one polar cell in addition to fewer border cells. Loss of Rap1 did not alter polar cell specification or the timely elimination of supernumerary polar cells during early oogenesis. Nor did Rap1 regulate activation of STAT in the follicle cells that will form the border cell cluster. Instead, during mid-oogenesis, Rap1 maintained the viability of anterior follicle cells including the mature polar cells. Without proper Rap1 activity, one or both anterior polar cells underwent apoptosis resulting in smaller border cell clusters. Notably, Rap1-deficient border cell cluster size was rescued by blocking cell death using the apoptosis inhibitor p35. These data together show that Rap1 regulates cell survival through blocking apoptosis, resulting in clusters with the optimal number of border cells. Remarkably, Rap1-deficient polar cell shape was drastically altered, potentially contributing to polar cell death. Thus, Rap1 is a new regulator of organizer cell viability that promotes polar cell survival and morphogenesis to build the migratory border cell collective.

596A Phosphoinositide PI(3,4,5)P3 turnover modulates cytoskeletal forces controlling *Drosophila* eye morphogenesis *Jacob Malin*, Christian Rosa-Birriel, Victor Hatini Tufts University School of Medicine, Boston, MA

Epithelial remodeling relies on modulation of mechanical forces that alter the shape and the relative arrangement of cells. In the *Drosophila* pupal retina, remodeling is dependent on the repeated pulsed contraction and expansion of cell-cell contacts at the level of adherens junctions (AJs). While actomyosin network assembly controls contraction, branched F-actin network assembly controls expansion. Our goal is to uncover mechanisms that control the pulsatile dynamics of the two networks and coordinate their activities. We previously found that levels of the phosphoinositide PI(3,4,5)P3 (PIP3) increase during contact expansion and decrease during contraction. This result suggests that PIP3 activates the WAVE regulatory complex (WRC) to control F-actin branching and protrusive membrane dynamics that drive expansion. To examine the system further, we investigated the role and localization of the phosphatase and tensin homolog (Pten) that hydrolyzes PIP3 to PI(4,5)P2 (PIP2), and separately phosphoinositide 3-kinase (PI3K), the enzyme that phosphorylates PIP2 to PIP3. We find that Pten localizes to AJs and accumulates dynamically during both expansion and contraction. The dynamic localization of PI3K is still being investigated. In *pten* mutant eyes, the dynamics of PIP3 and of protrusive and contractile effectors are disrupted and cell-cell contacts are shortened or lost altogether. Loss of *pten* and constitutive activation of PI3K produce similar phenotypes, and *PI3K* depletion induces related phenotypes. Overall, our findings suggest that the cyclical production and hydrolysis of PIP3 is central to the control and coordination of contractile and protrusive dynamics. PIP3 has been previously implicated in controlling actomyosin contractility and endocytosis in epithelial remodeling. Our findings suggest that PIP3 turnover, rather than overall PIP3 levels, is required to fine-tune contractile and protrusive dynamics that shape this epithelium during development.

597B Customization of tissue growth coordinates organ form and function in the embryo *Rajprasad Loganathan*[1], Daniel Levings[2], Ji Hoon Kim[1], Michael Wells[3], Hannah Chiu[1], Yifan Wu[1], Matthew Slattery[2], Deborah Andrew[1] 1) Johns Hopkins University; 2) University of Minnesota Medical School; 3) Idaho College of Osteopathic Medicine

Developmental tissue growth in the *Drosophila* embryo has hitherto been ascribed exclusively to the contributions from early mitotic cycles. Post mitotic non-proliferative growth of embryonic tissues has, therefore, been considered negligible for organogenesis. In this work, we describe a significant contribution of non-proliferative cell growth to the formation of two embryonic tubular organs—the salivary gland (SG) and the trachea. The BTB-domain transcription factor Ribbon (Rib) plays a critical role in promoting growth of both organs. In the SG, loss of Rib results in abnormal cell shape and a significant reduction in cell size, attributable to a loss of cytoplasmic volume gain during embryogenesis. In the trachea, Rib loss also results in a significant reduction in cell volume and a failure of segmental branch connectivity. Tissue-specific ChIP-Seq revealed that, in the SG, Rib binds almost all SG-expressed ribosomal protein genes (RPGs). Follow-up transcription assays (RT-qPCR and FISH) revealed that Rib is required for high levels of expression of all ten of the RPGs we have so far tested. Although Rib can directly bind SG RPG enhancers in vitro, the binding is both weak and not sequence selective. We have demonstrated, however, that Rib may attain specificity for RPG enhancer binding and transcriptional activation through direct cooperative interactions with three previously known activators of RPG expression—Trf2, M1BP, and Dref. Rib, in addition to binding SG RPGs, also binds translation factors and chaperones (other components of the “translatome”) implicated in protein synthesis. The mechanism by which Rib-dependent SG cell growth occurs—boosting expression of RPGs and other components of translation—suggests functional consequences beyond cell growth, *i.e.*, priming this organ for its secretory function by enriching the translational machinery to coordinate organ form with function. Surprisingly, Rib binds non-ribosomal genes to promote cell growth in the embryonic trachea where tissue growth is geared primarily to meet the demands of branch connectivity rather than secretion. The differential deployment of cell growth mediators in these two tissues by a single transcription factor

suggests a model in which the growth of individual tissues is customized to coordinate organ form with function.

598C Investigating the role of Uif and Gprk2 in tissue-specific growth of the larval trachea *Zihao Yu*, Robert Ward
Case Western Reserve University

Most animal species show allometric growth, which means that different organs and tissues grow at different rates relative to each other. Understanding the mechanisms of how different tissues grow is important, since it can provide insight about unique signals and pathways required for development of different organs. The Drosophila larval trachea is an excellent model for tissue-specific growth, since larvae are transparent and the trachea can be easily visualized. In addition, we and other labs have identified a small number of genes that are required for growth specifically in the larval trachea. Two of these genes are *uninflatable* (*uif*) and *G-protein coupled receptor kinase 2* (*Gprk2*). *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 have the relative size of that found in wild type larvae. *Gprk2* encodes the only non-neuronal G-protein receptor kinase encoded by Drosophila. Loss of Gprk2 also results in larval lethality, but the mutant larvae have long and highly convoluted trachea. In other developmental contexts, Gprk2 is required to phosphorylate G-protein coupled receptors to mark them for endocytosis and functional attenuation. Preliminary results indicate that Uif levels are higher in *Gprk2* mutant larval trachea, raising the possibility that Gprk2 may promote the endocytosis of Uif. We are looking at the genetic and biochemical interactions between *uif* and *Gprk2*, and their effect on growth through the insulin signaling pathway.

599A Regulated actomyosin turnover is essential for eye epithelial morphogenesis *Christian Rosa*, Jacob Malin, Victor Hatini Tufts University

Epithelial morphogenesis is fundamental to animal development and the understanding of congenital disorders. In the fly retina, the repeated assembly and disassembly of contractile actomyosin and branched F-actin networks drive epithelial morphogenesis. However, the roles and the mechanisms that control the pulsatile dynamics of each network are poorly understood. The Rho1 RhoGTPase regulates the assembly of the contractile actomyosin network by activating both the formin Diaphanous (Dia) to promote the assembly of linear actin filaments and non-muscle Myosin II (MyoII) to bind and contract these filaments. We hypothesized that actomyosin turnover is essential for rebalancing forces in the epithelium for proper remodeling and for preventing tissue damage. To test this idea, we overexpressed a constitutively active myosin light chain kinase (MLCK.ct) and separately a constitutively active Dia (Dia.ca) to respectively activate either MyoII or the assembly of linear F-actin. Both manipulations led to defects in epithelial remodeling. MLCK.ct inhibited pulsatile actomyosin assembly and induced ruptures in the epithelium. Likewise, Dia.ca disrupted epithelial remodeling. To determine the mechanisms that regulate actomyosin turnover, we searched for RhoGEFs and GAPs affecting epithelial remodeling. We identified RhoGEF2 and RhoGAP71E as regulators of actomyosin turnover in this process. RhoGEF2 localized medioapically, while RhoGAP71E localized both medioapically and at the cell surface. Genetic analysis combined with live imaging revealed that RhoGAP71E inhibits pulsatile actomyosin dynamics and decreases MyoII levels, while RhoGEF2 has opposite effects. Our data suggest that RhoGEF2 and RhoGAP71E activate and inhibit Rho1, respectively, to sustain actomyosin turnover, promote proper remodeling and rebalance forces in the tissue to inhibit tissue rupture.

600B Anisotropic Myosin Recruitment Responds To A Static Source During Drosophila Body Axis Elongation *Matthew Lefebvre*, Nikolas Claussen, Noah Mitchell, Sebastian Streichan University Of California Santa Barbara

Morphogenesis is an inherently dynamic process which bridges cellular and tissue scales. Across metazoans, the actomyosin cytoskeleton plays a central role in sculpting morphogenetic geometry. Yet the mechanisms by which it is controlled remain poorly understood. Recent evidence suggests two regulatory principles: instructive genetic programs, and a dynamic response to mechanical stimuli. The two are not mutually exclusive and have proven difficult to disentangle. In \emph{D. melanogaster} body axis elongation, a canonical example of convergent extension, both the required genetic patterning inputs, and the force-generating cytoskeletal actors have been identified. Non-muscle myosin II (MyoII) is recruited to dorso-ventral cell-cell junctions where it generates forces. In anterior-posterior patterning mutants axis elongation is impaired, but the way in which genetic information orients the cytoskeleton remains unclear. We study this process using live \emph{in-toto} imaging and dynamic, quantitative analysis. A systematic comparison of the genetic patterning system as well as the magnitude and orientation of junctional MyoII reveals a series of discrepancies. Crucially, during the course of axis elongation, gene patterns permanently deform with the flowing tissue, while the MyoII orientation remains aligned to the dorso-ventral axis, with only a temporary deflection. Therefore, we propose that MyoII is recruited by a geometrically defined static source rather than cell-intrinsic genetic patterning. Under this hypothesis, we can explain and quantitatively predict MyoII dynamics.
Our results suggest that genetic patterning does not directly instruct cytoskeletal regulation during axis elongation. Mechanical cues, in particular embryo-scale tension, are a natural candidate for the static source whose signatures we uncover, since they need not behave in the same way as genetic cues in deforming tissue. Our results add a novel perspective on developmental biology: morphogenesis may be organized by tissue geometry without constant input of

genetic patterning, allowing for robust, modular and self-organized processes.

601C ArfGAP1 regulates collective cell migration *in vivo.* *Alison Boutet*[1,2], Carlos Zeledon[1,2], Xiaojuan Sun[3], Gregory Emery[1,2] 1) Institut de recherche en immunologie et en cancérologie, IRIC, Montréal, Canada; 2) Université de Montréal, Montréal, Canada; 3) School of Basic Medical Sciences, Henan University, Kaifeng, China

Introduction: Collective cell migration plays important roles in morphogenesis and embryonic development and is a main feature of the formation of metastases in several cancers. Unlike single cell migration, collective cell migration is characterized by cell-cell adhesion and cell-cell communication. We have previously demonstrated that vesicular trafficking plays a critical role in cell guidance and cell-cell communication during collective cell migration. A recent screen aimed at identifying new regulators of vesicular trafficking involved in collective migration identified ArfGAP1 as a regulator of border cell migration in the *Drosophila* ovary.

Methods and Results: Between stages 9 and 10 of the *Drosophila* egg chamber development, the so-called border cells form a cluster that is attracted by the oocyte through the secretion of ligands to receptor tyrosine kinases (RTKs). We found that the depletion of ArfGAP1 specifically in border cells induces migration defects. Indeed, clusters devoid of ArfGAP1 are able to initiate their migration, but loose directionality. Investigating the cause of this phenotype by analyzing various determinants of border cell migration, we found that the depletion of ArfGAP1 reduces the level of active RTKs at the plasma membrane as they accumulate in late endosomal compartments. Looking further in the degradative pathway, our results indicate an increase of active RTKs inside late endosomes, combined with an increase in Rab7 signal and lysosomes in border cells depleted of ArfGAP1. Our results suggest that ArfGAP1 is necessary for the proper sorting of active RTKs in endosomes to maintain RTKs at the plasma membrane and allow directionality. Moreover, rescue experiments and genetic interactions revealed that the role of ArfGAP1 in border cell migration is dependant of its GAP activity and could act through Hrs and Lrrk, two proteins involved in multivesicular body formation and RTKs degradation.

Conclusion and Relevance: We identified ArfGAP1 as a new regulator of border cell migration that might acting through vesicular trafficking to maintain of active receptor tyrosine kinases at the plasma membrane. This study could potentially reveal a new important mechanism in collective cell migration, and by extent in cancer dissemination.

602A Uncovering the mechanism of hematopoietic niche formation *Kara Nelson*, Stephen DiNardo University of Pennsylvania Philadelphia, PA

Niches have been well-established as a source for important extrinsic components that regulate the balance between progenitor cell maintenance and differentiation in tissues. Most well-studied niches are positioned reproducibly within tissues, and are comprised of an organized collective of cells. This implies that formation of niche structure is regulated, and suggests that the tissue-specific structure of niches is pertinent to the underlying biology of the tissue that the niche supports. However, mechanisms that determine the initial organization of cells constituting a niche, and the location of the niche within a tissue remain less-understood. To investigate mechanisms of niche formation, we use the posterior signaling center (PSC)—the niche of the larval lymph gland—as a model niche. The PSC niche maintains stem cell-like hematopoietic progenitors that eventually differentiate into mature hemocytes that respond to immune challenges and infliction of wounds, and replenish hemolymph during homeostasis. Initial formation of the PSC occurs during embryogenesis: PSC cells are specified laterally during mid-embryogenesis, and in late embryos, the PSC resides at the dorsal surface of the embryo, coalesced at the posterior of the lymph gland. Preliminary live-imaging of PSC formation revealed that alary muscle (AM) and visceral mesoderm (vm), two tissues nearby the PSC, move towards the dorsal midline in concordance with the PSC. Thus, we hypothesized that one of these tissues, AM or vm, sends guidance cues that direct PSC migration towards the dorsal midline/lymph gland posterior. Using tissue-specific ablation of the AM, we demonstrate that it is not required for PSC formation. However, analysis of late-stage mutants that lack visceral mesoderm shows that fewer PSC cells are present at the lymph gland posterior, suggesting a requirement for vm in the formation of the PSC. Future experiments will investigate if, in the absence of vm, fewer PSC cells were specified, or some PSC cells underwent apoptosis, or PSC cells were misplaced within the embryo during migration – the latter of which would suggest that vm sends a directional or competency cue important for PSC cell migration.

603B *Transcriptome analysis reveals temporally regulated genetic networks during border cell collective migration.* *Emily Burghardt*[1], Jessica Rakijas[1], Antariksh Tyagi[2], Pralay Majumder[3], Bradley J.S.C. Olson[1], Jocelyn A. McDonald[1] 1) Kansas State University; 2) University of North Dakota; 3) Presidency University, Kolkata, India

Collective cell migration underlies many processes essential to the life of an organism, 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 help them move rapidly through the surrounding tissue. Later, as migration slows down, the cluster rotates several times, then stops at the oocyte border. Successful border cell migration relies on cell signaling, polarization of the cluster, including directed protrusions, remodeling of the actin cytoskeleton, 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 the migration of border cells. 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. Thus we wanted to identify genes whose expression changed during border cell migration. We performed RNA-sequencing on border cells specifically isolated at pre-, mid-, and late-migration stages. Transcriptome analyses of these cells showed that 1,794 transcripts (1,394 unique genes) were significantly differentially expressed during border cell migration. Downstream analyses, including clustering of genes by expression patterns and testing for gene ontology enrichment and genetic networks, identified nine groups of differentially expressed genes. Many of these genes have known roles in cell junction assembly, epithelial differentiation, cellular morphogenesis, the actin cytoskeleton, and epithelial-to-mesenchymal transitions, but also metabolic processes. To validate this RNA-sequencing approach, we confirmed the expression and/or function of a subset of these genes in border cells, including *CG11147, CHES-1-like,* and *Arf51F.* Thus, our transcriptome analysis identified differentially expressed genes in migrating border cells and highlighted multiple genetic networks, as well as individual genes, that may function in border cell migration.

604C Coordination of border cell cohesion through localization of the RacGEF Cdep by the scribble complex. *Joseph Campanale*, James Mondo, Denise Montell University of California, Santa Barbara

Collective migration of numerous cell types including neural crest, endothelial, epithelial, tumor and stromal cells contributes to normal development, wound healing, angiogenesis, and tumor metastasis. The study of multiple *in vitro* and *in vivo* models has revealed mechanisms promoting collective polarization, motility, direction-sensing, and adhesion. Border cells in the Drosophila ovary serve as a useful *in vivo* model for collective cell migration. Border cells are a group of four to six migratory cells that surround and carry two non-migratory polar cells in between nurse cells in developing egg chambers. Here we report that cryptic basal protrusions in border cells ensure cluster cohesion. We further show that the basolateral complex protein scribble promotes cryptic protrusions by localizing LGL and the Rac guanine nucleotide exchange factor Cdep to membranes. The finding that membrane targeting of Cdep is sufficient to suppress scribble knockdown suggests that Cdep is a major effector for the basolateral complex. Additionally, Scribble restricts cryptic protrusions from encroaching onto the apical domain, which is essential for proper docking of the cluster apical surface to the oocyte at the end of migration. This work suggests novel roles for cryptic protrusions, Scribble, and Cdep in the collective and cooperative movement of a heterotypic cell group in vivo.

605A Investigating the role of Ecdysone signaling during mid embryogenesis using Halloween genes *Jae Ho Lee*, Riti Mital, Robert Ward Case Western Reserve University

20-hydroxyecdysone (20E) is a well-characterized steroid hormone required for major development changes in Drosophila. 20E surges before each larval molt, before pupariation, and during terminal differentiation of the adult structures. During molting and metamorphosis, 20E binds to its receptor to directly activate a group of early genes such as Broad-Complex (BR-C), E74 and E75, which activate late genes that are performing cell death of obsolete larval tissues, cell proliferation, etc. There is also a less well characterized pulse of 20E during mid embryogenesis. Previous studies indicate a role for 20E signaling in germband retraction, head involution, dorsal closure, and cuticle secretion. To gain a mechanistic understanding of the function of the 20E during embryogenesis, we are characterizing two Halloween mutants, *disembodied* (*dib*) and *shroud* (*sro*). These genes encode biosynthetic enzymes required for 20E synthesis. Using confocal microscopy of fixed and live wild-type and mutant embryos, we are examining the signaling and cytoskeletal dynamics required for dorsal closure. In addition, we conducted RNA sequencing of wild-type and mutant 9- and 13-hour embryos and found approximately 2000 genes that are differentially expressed between wild-type and mutant embryos. Gene ontology analysis identified genes involved nucleic acid binding and transferase activity to be significantly over represented in this collection. Interestingly, among the top 50 20E-induced genes, 11 encode Inc RNA or Antisense RNA. To extend this study, we are using RNA interference and loss-of-function mutants of 20E induced genes to investigate their role in dorsal closure.

606B Regulation of epithelial tissue sealing during Drosophila dorsal closure by the PI4P phosphatase Sac1 *Kimberley Gauthier*[1], Julie Brill[1,2] 1) Cell Biology Program, The Hospital For Sick Hospital, Toronto, Canada; 2) Department of Molecular Genetics, University of Toronto, Toronto, Canada

A recurring theme throughout development and epithelial tissue morphogenesis is the sealing or fusion of epithelial tissues, forming new cell junctions and a single seamless layer of cells. This process is central in wound healing, and dysregulation can lead to developmental anomalies such as cleft palates and neural tube defects. Epithelial sealing is essential in dorsal closure during Drosophila embryogenesis in which the epidermis wraps around the developing embryo, covering the underlying epithelium (the amnioserosa) and rejoining at the dorsal midline where the tissue seamlessly zippers shut. Dorsal closure is driven by actomyosin contractility in the amnioserosa, Jun kinase (JNK)

signaling in the leading edge cells of the epidermis, and formation of a supracellular actin cable around the dorsal hole. Previously, embryos with *Sac1* mutations were found to die during embryogenesis, leaving behind gaping dorsal anterior holes. Sac1 is a lipid phosphatase that dephosphorylates phosphatidylinositol-4-phosphate (PI4P), a lipid needed for membrane trafficking from the Golgi. *Sac1* mutant embryos also have ectopic JNK activation and excessive cellular constriction in the amnioserosa. How Sac1 regulates JNK signaling and actomyosin contractility during dorsal closure remains unknown. Using time-lapse confocal microscopy to image a fluorescent myosin regulatory light chain marker, I found that dorsal closure proceeds at a slower pace in *Sac1* mutants, and that some embryos develop tears in the amnioserosa and epidermal puckering towards the final stages of dorsal closure. In ongoing studies, I will further characterize the role of Sac1 in regulating epithelial sealing, actomyosin contractility, and JNK signaling activation. My results will uncover how Sac1 and phospholipid homeostasis orchestrate contractile forces and cellular signaling to promote tissue morphogenesis.

607C Smog GPCR regulates distinct myosin pools and cortical actin organization during *Drosophila* SG invagination *Vishakha Vishwakarma*, Thao Phuong Le, SeYeon Chung Louisiana State University

Epithelial tube formation requires Rho-dependent actomyosin contractility that generates 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* embryonic salivary gland (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 for Fog that translates Fog signal to cytoskeletal reorganization has not yet been identified. 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 maintaining epithelial integrity. *smog* loss results in reduced/mislocalized junctional myosin, which correlates with reduced and discontinuous signals of the apical determinant protein Crb and the key adherens junction protein E-Cad; this leads to a distorted embryonic morphology with an abnormally elongated SG placode. Moreover, *smog* null mutants also show enhanced bleb formation in the apical domain of SG cells during invagination, suggesting disorganized cortical actin networks upon *smog* loss. Genetic interaction tests with actin polymerizing/depolymerizing factors reveal that Smog regulates the cortical actin networks by regulating actin assembly/disassembly. Our study supports a model that Smog regulates apicomedial and junctional myosin pools in a Fog-dependent and -independent manner, respectively, and reveals a new role of Smog in regulating cortical actin organization during epithelial tube formation.

608A Snail drives epithelium-to-mesenchymal transition by cytoplasmic sequestering of polarity protein Bazooka/Par-3 *mo weng*[1], Rolin Sauceda[1, 2] 1) University of Nevada, Las Vegas; 2) Stanford University School of Medicine

Epithelial-to-mesenchymal transition(EMT) converts cells from highly connected and apicobasally polarized epithelial states into detached and often migratory mesenchymal states. It is an essential process during development and its misregulation underlies tumorigenesis. In the conventional model, a key step in EMT is to eliminate cell-cell junctions through transcriptional repression by EMT transcription factors, such as the conserved EMT driver Snail. However, recent advances suggest most EMTs display reversible intermediate states and do not reach the final mesenchymal state where junctions are completely lost. We show previously that in *Drosophila* gastrula, Snail drives EMT in mesoderm through a post-transcriptional mechanism: disassembling junctions through downregulation of polarity protein Bazooka/Par-3. Here we show that this downregulation of Bazooka/Par-3 is through a cytoplasmic sequestering of Bazooka/Par-3 proteins. Snail is necessary and sufficient in downregulating cortical Bazooka/Par-3. Despite the loss of cortically localized Bazooka/Par-3, we do not observe significant changes in Bazooka/Par-3 protein levels upon Snail overexpression in embryo extracts. To examine the change in cytoplasmic levels of Bazooka/Par-3 in response to Snail expression, we induced Snail overexpression clones in egg chamber follicle epithelium which normally do not express Snail. We find that the Snail-dependent downregulation of cortical Bazooka/Par-3 is accompanied by an increase in cytoplasmic levels of Bazooka/Par-3 proteins. This suggests Baz is regulated at post-translational levels and preferentially localizes in cytoplasm. In addition to the loss of Bazooka/Par-3 and adherens junctions, Snail expression in these cells also leads to cell flattening and even delamination from the follicle epithelium. Overall, these results suggest a post-translational pathway is downstream of Snail in regulating Bazooka/Par-3 localization and it provides a possible molecular mechanism for EMT intermediate states.

609B Physical aspects of *Drosophila* gastrulation *Konstantin Doubrovinski*[1], Joel Tchoufag[2], Mohamad Ibrahim Cheikh[1], Kranthi Mandadapu[2], Amanda Goldner[1] 1) UT Southwestern; 2) UC Berkeley

Epithelial morphogenesis is a process through which epithelia develop complex shapes. A common mode of epithelial morphogenesis is folding, where an initially flat sheet of cells bends out of plane to create a fold. A popular system to study folding is ventral furrow formation during gastrulation in *Drosophila melanogaster*. Despite a long-standing effort to understand the mechanism of tissue folding, the mechanism of ventral furrow formation remains unclear.

We argue that the key missing information required to understand tissue folding is the knowledge of tissue material properties. Indeed, as is known from basic physics considerations, the knowledge of tissue material properties together with the knowledge of tissue deformation dynamics determine the forces driving the dynamics uniquely. Hence, determining material properties of embryonic tissues is absolutely required to arrive at a predictive model of morphogenetic dynamics.
Here, we present a novel technique based on micron-sized bendable cantilevers to directly probe material properties of epithelia in a developing embryo. Our data characterizes how embryonic tissues respond to localized nanonewton range forces. In particular, we show that (1) the tissue restores its shape incompletely after an external pulling force is removed, (2) the tissue restores its shape to a lesser extent if the applied force is spread over a larger area, (3) when constant force is applied, the displacement of the point being pulled increases according to a characteristic 1/2 power law. To interpret our data, we developed a novel 3D computational model of epithelia. Based on a very small set of simple assumptions, our model quantitatively explains all the above as well as several other key features of our measurements.
Our modeling approach can be used further to explain key aspects of tissue dynamics during *Drosophila* gastrulation. Specifically, we characterize gastrulation dynamics in a genetic background (anillin knockdown) where cells remain completely open to the yolk-sack. Despite cells being completely open on their basal site, ventral furrow formation proceeds (almost) completely unperturbed, with ventral furrow forming to the full extent. Our computational model readily accounts for this unexpected observation. In this way, our *in vivo* measurements together with computational modeling strongly constrain the relative contributions of the various physical effects involved in ventral furrow dynamics.

610C Investigating a morphogenetic role for septate junction proteins in cell shape changes and polarity during dorsal closure *Oindrila De*, Robert Ward Case Western Reserve University

Septate junctions (SJs) form an occluding barrier in invertebrate epithelia. More than 30 SJ constituents have been identified and characterized for their canonical barrier function but some studies have highlighted a non-occluding role for SJ genes in *Drosophila* morphogenesis. In our previous studies, we demonstrated a non-barrier requirement for a subset of core SJ genes during head involution, dorsal closure, and salivary gland development in the fly embryo. However, the mechanistic nature of this requirement is unknown. To address this, we are using dorsal closure (DC) as our model to investigate the morphogenetic function of core SJ genes. DC is a mid-embryogenesis process that seals an epidermal gap, which occurs as a result of germ band retraction. It 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. Additionally, cells of the lateral epidermis elongate dorsoventrally and exhibit planar polarized expression of various molecular components including actin, tubulin, and Rab GTPases. Loss-of-function mutations in core SJ genes, including *coracle*, *Macroglobulin complement-related*, and *Neurexin-IV* result in a failure to complete DC and is associated with a dorsal open phenotype in cuticle preparation of embryos. Here we are characterizing cellular defects in SJ mutant embryos that may contribute to their failure in DC. We examined fixed tissue of SJ mutants and observed a lack of a smoothly organized leading-edge during advanced stages of DC. Also, lateral epidermal cells in SJ mutants show significant defects in cell shape, including a lower aspect ratio, reduced area, and increased circularity of their apical surfaces. Additionally, SJ mutants fail to maintain robust organization of microtubules and actomyosin in the epidermis, amnioserosa, and leading-edge towards the end of DC. We are using live confocal imaging to quantify defects in actomyosin dynamics at the leading-edge and amnioserosa in SJ mutants. We are analyzing SJ mutants for subtle alteration in apical-basal polarity of the dorsal epidermal cells that may affect cell shape. We are also examining SJ mutants for defects in localization of Rab GTPases, which may cause reduced polarized trafficking of cytoskeletal and adhesion markers, resulting in defects in cell shape changes and ultimately leading to a defective DC.

611A Molecular players of mis-specified cell elimination during development *Menna El Gammal*, Olga Klipa, Fisun Hamaratoglu Cardiff University

Elimination of aberrantly specified cells is vital for development and the prevention of tumour formation. While our cells carry the same genetic information, only a subset of genes are expressed in a certain cell type, determining its fate. Normal organ development heavily relies on the maintenance of correct gene expression patterns in the different cell types within it. Genetic or epigenetic mutations may cause mis-expression of fate-determining factors in cells, disrupting proper patterning and compromising organ function. It is therefore important for a mechanism to be in place to detect and remove those harmful cells. Importantly, the same strategy may be accountable for recognizing and eliminating early cancer cells with incorrect fates. However, little is known about the responsible molecular players. To elucidate this, we induced aberrantly specified patches of cells by modifying the levels of the transcription factor Apterous in Drosophila. This was done by mis-expressing its negative regulator dLMO. Apterous is expressed in the dorsal compartment of the imaginal wing disc, where it defines dorsal fate. We have since undergone 3 screens. First, we identified genes which are differentially expressed in the context of mis-specified cell elimination using RNA-seq. Then, we tested their involvement in the process with two RNAi verification screens, where we observed any potential changes in adult fly wings and larval wing discs. We reasoned that if a gene is required for aberrant clone elimination, then its downregulation would rescue

those clones, leading to wing defects. In this way, we identified a set of genes that are likely involved in the process of mis-specified cell elimination.

612B Exploring the function of Canoe's intrinsically disordered region in linking cell junctions to the cytoskeleton during morphogenesis *Rachel Szymanski*, Noah Gurley, Zuhayr Alam, Mark Peifer University of North Carolina at Chapel Hill

The remarkable ability of cells to change shape and move without disrupting tissue integrity is a hallmark of embryonic development. To do so cells must link the contractile actomyosin cytoskeleton to cell-cell and cell-matrix junctions, and this linkage must be robust and dynamic. We have focused on the multidomain scaffolding protein Canoe, homolog of mammalian Afadin, which links the cadherin-catenin complex to actin. It includes two N-terminal RA domains, which bind the small GTPase Rap1, followed by FHA, Dilute and PDZ domains. These are separated from the C-terminal F-actin binding domain by a long intrinsically disordered region (IDR). IDRs are now recognized as important players in the multivalent interactions that assemble multiprotein complexes, including those that form phase-separated biomolecular condensates. One of our tasks is to define Canoe's mechanistic role, by taking it apart as a machine. We began by exploring the roles of its PDZ and F-actin binding domains, thinking they provided the direct linkage between cadherin and actin. However, deleting each domain had only modest effects on protein function. We now have returned to this analysis, exploring a surprising result from earlier work—protein null alleles of *canoe* have a milder zygotic phenotype than the "canonical" *canoe* alleles, whose defects in dorsal closure gave the gene its name. To explore this, we have sequenced a series of 22 EMS-induced *canoe* alleles. Most result from premature stop codons, arrayed across the coding sequence. Intriguingly, the two strong "canonical" alleles carry stop codons early in the IDR. We suspect they encode truncated proteins that act in a dominant negative fashion, interfering with maternally-contributed Canoe protein. We are now exploring the function of this diverse set of Canoe proteins, examining both zygotic and maternal/zygotic mutants. We think this will help define the role of Canoe's IDR in protein function.

613C Defining the roles of the small GTPase Rap1 and its regulator Dizzy in embryonic morphogenesis *Kristi Yow*, Kia Perez-Vale, Noah Gurley, Mark Peifer University of North Carolina at Chapel Hill

During embryonic development, epithelial cells must change shape and move while maintaining a robust linkage between one another. In *Drosophila*, multiple molecular mechanisms are involved in maintaining this linkage between adherens junctions and the cytoskeleton. Our lab has focused on the role of the junction-cytoskeletal linker Canoe, which regulates diverse events in embryonic morphogenesis, from apical-basal polarity establishment to apical constriction to convergent elongation and collective cell migration. Canoe is activated by the small GTPase Rap1, which in turn is regulated by diverse Guanine nucleotide exchange factors (GEFs). We and others have defined the roles Rap1 plays during cellurization and mesoderm invagination, but little is known about its role in later embryonic morphogenesis. We are thus exploring the roles of Rap1, and those of one of its GEF regulators, Dizzy, contrasting these with roles of the Rap1 effector Canoe. We used maternally driven shRNAi to deplete both maternal and zygotic Dizzy. Our data reveal Dizzy is required to reinforce junctions under tension during germband extension, thus making Dizzy loss comparable to *canoe* maternal/zygotic mutants. Dizzy knockdown also altered junctional planar polarity, though not to the same extent as Canoe. Later in development the *dizzy* mutant epidermis remains relatively intact, with the most severe defects localized to the ventral epidermis, as seen previously in *canoe* mutants. Together these data suggest that Dizzy is the predominant GEF regulating Canoe via Rap1. We're now extending this analysis to explore the role of Rap1 in morphogenetic movements. Our preliminary data suggest that *Rap1* mutants have substantially more severe defects in epithelial integrity. Cell shapes are altered beginning at the onset of germband extension. The balance of apical contractility between different cells appears to be lost, leading to epithelial folding, and by the end of germband extension epithelial junctions become fragmented in many cells. These data suggest Rap1 must have other effectors in addition to Canoe—intriguingly, the *Rap1* phenotypes are more similar to those of embryos lacking both Canoe and Pyd (fly ZO-1) function. These data also suggest additional GEFs must regulate Rap1 during these stages. Together, our data provide new insights into the mechanisms regulating cell shape change and ensuring tissue integrity.

614A Protein biogenesis factors Nascent Polypeptide Associated Complex–*alpha* and Signal Recognition Particle are required in heart development *Analyne Schroeder*, Georg Vogler, Alexandre Colas, Rolf Bodmer Sanford Burnham Prebys Medical Discovery Institute, La Jolla, CA

Congenital Heart Disease (CHD) is driven by a strong genetic predisposition, yet only a small subset of patients (~20%) are diagnosed with a precise genetic cause. Therefore, expanding the pool of genes associated with CHD and establishing the functional relationships between genes can assemble a more comprehensive genetic network to better understand cardiac development and pathogenesis. In our studies, we identified protein biogenesis cofactors Nascent polypeptide Associated Complex (NAC) and Signal Recognition Particle (SRP) that bind disparate subsets of emerging nascent polypeptides at the ribosome exit site to direct polypeptide fates, as novel regulators of cell differentiation and cardiac morphogenesis. Knockdown (KD) of the α- *(Nacα)* or β- subunit (*bicaudal)* of NAC in the developing *Drosophila* heart led to disruption of cardiac remodeling during pupal stages resulting in an adult fly with no heart. Heart loss was

rescued by combined KD of *Nacα* with the *hox* gene *abd-B.* This genetic interaction between *hox* genes and *Nacα* was recapitulated in differentiation assays using human Multipotent Cardiac Progenitors (MCPs). KD of *Nacα* in MCPs led to decreased cardiomyocytes (CM) and increased fibroblasts (Fib) which were reversed upon co-KD with mammalian *hox* genes *HOXC12 and HOXD12*. KD of cardiogenic transcription factors (TFs) *Gata4/6* and *MyoCD* led to different population growth profiles, with decreased CMs but no change in Fibs, indicating that *Nacα* may utilize distinct mechanisms in driving cell fates compared to these TFs. The effect of *Nacα* KD on the fly heart was temporally regulated, in that KD in embryo or in pupae caused only a partial loss of the heart, whereas KD during both stages led to heart loss similar to continuous KD throughout life. This suggests that embryonic *Nacα* KD may in part reprogram cells leading to aberrant cardiac remodeling during pupal stages. Lastly, KD of several SRP subunits individually in the fly heart produced a range of cardiac phenotypes that targeted specific segments and cell types, indicating spatially regulated activities of SRP components in the heart. Together, these data suggest that despite NAC and SRP ubiquitous presence, they displayed spatially and temporally fine-tuned activities for proper cardiac morphogenesis. *Nacα's* interaction with cardiac-specific *hox* gene functions builds upon the novel role of this pathway and expands our understanding of the complex genetic networks involved in cardiac development and pathogenesis.

615B The role of Akirin/NuRD interactions during heart development *Mia Jones*, Hayley Milner, Scott Nowak Kennesaw State University

Congenital heart defects are the most prevalent birth defect presented in the human population. A number of these cases have been ruled as sporadic, or resulting from different interactions of many independent genetic loci and alleles. 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. In *Drosophila*, the process of heart specification and patterning is controlled by a number of transcription factors that work together with the nuclear co-factor, Akirin, to mediate cardiac gene expression. Preliminary work in the Nowak Laboratory found that Akirin likely regulates gene expression by working together with the Nucleosome Remodeling and Deacetylase (NuRD) complex. Embryos bearing mutations in different NuRD subunits produce hearts, but they are often severely misshapen, poorly patterned, and have reduced numbers of cardiomyoblasts. Moreover, quantitative live imaging of cardiac contractions in pre-hatching embryos indicates that these hearts display impaired cardiac function. Together, these data suggest that Akirin/NuRD interactions may be key regulators of proper heart function.

616C Tissue scale viscoelastic properties influence 3-D organ morphology in the developing fly retina *Jacob Decker*, Xiao Sun, Ilaria Rebay University of Chicago

Development of functional tissue morphologies requires precise spatiotemporal regulation of cellular processes that generate mechanical forces and modulate tissue material properties. Extensive work has revealed the existence of common cell biological strategies that are employed to shape epithelial sheets; however, the influence that tissue scale material/viscoelastic properties impart on morphogenetic processes is not understood. There is growing evidence that within a single cell, different organization states of the cytoskeleton result in different cellular viscoelastic properties, but how these cellular behaviors—in aggregate—influence tissue-scale material properties, is an open question. The Drosophila retina, which has long been used as a model system to understand the cell biological basis of tissue patterning, presents an intriguing opportunity to explore the cell biological and biophysical processes that coordinate to shape the fly eye at the tissue scale.

The fly retina is a neuroepithelium comprised of ~750 multicellular units called ommatidia, each of which comprises a core cluster of photoreceptor neurons surrounded by a supportive lattice of non-neuronal cells. During development, ommatidial units are positioned in a stereotyped curvature that is critical for the optical resolution of the adult compound eye. During pupal development, the apical surfaces of the photoreceptor cells involute and then elongate along the optical axis of the tissue in a process called apical expansion. We have recently discovered that the retina establishes its curvature prior to apical expansion onset, rather than concomitantly as it was previously assumed. This suggests that retinal curvature establishment is a discrete morphogenetic process driven by a distinct mechanism. Interestingly, coincident with curvature formation, the retinal epithelium reorganizes apical cell contacts and cell adhesion machinery, resulting in precise hexagonal packing of ommatidia. Live-imaging experiments of retinal epithelia during this period suggest that the tissue undergoes a transition from a viscous-fluid like state to an elastic-solid like state. This transition depends on both packing geometry of ommatidia in the epithelium and actomyosin contractility. This newly described morphogenetic process in the pupal retina provides a model to probe the relationship between changes in tissue material properties and final tissue morphology. Quantitative live-imaging coupled with perturbations to cytoskeletal networks in the developing retina can provide mechanistic insight into the cellular processes that tune tissue material properties during tissue morphogenesis.

617A Characterization of mechanosensitive regulation of cell adhesion by membrane kinase Gish *Reina Koran*, Mo Weng University of Nevada Las Vegas

Mechanical input, much like a biochemical signal, can be converted into a biological response and trigger downstream effects. Adherens junctions, the major cell-cell junctions that resist physical tension, often mediate mechanosensitivity. Our lab has shown that in the mesoderm precursor epithelium of Drosophila gastrula, adherens junctions are strengthened in response to physical tension generated by apical myosin contraction. Such mechanosensitive junction strengthening is essential for mesoderm morphogenesis, but the molecular mechanism mediating this regulation is unknown. We have identified Gilgamesh (Gish) as a potential novel mechanosensitive junction regulator. Using multiple approaches, we show that Gish appears to be recruited to junctions in mesoderm upon Myosin activation but not in tissues without active Myosin. This recruitment is disrupted in alpha-catenin mutants, suggesting its dependency on adherens junctions. Importantly, loss of function of Gish leads to severe defects in adherens junctions and mesoderm internalization. Consistent with weakened adherens junctions, Gish mutants phenocopy junction mutants in membrane morphology. These data suggest Gish is a critical player in mechanosensitive remodeling of adherens junctions.

618B Quantiative Models of Mechanical Feedback in Morphogenesis *Nikolas Claussen*[1], Matthew Lefebvre[1], Hannah Gustavson[1], Noah Mitchell[1], Stefano De Renzis[2], Boris Shraiman[1], Sebastian Streichan[1] 1) University of California, Santa Barbara; 2) European Molecular Biology Laboratory, Heidelberg

Morphogenesis is an inherently dynamic process which bridges cellular and tissue scales. Quantitative analysis and mathematical modelling applied to dynamic imaging data can play a crucial role in elucidating developmental processes and can turn biological hypotheses into quantitative, testable predictions. The actomyosin cytoskeleton plays a central role in sculpting the developing embryo across metazoans, yet the mechanisms by which it is controlled are poorly understood. Recent evidence suggests that the cytoskeleton can dynamically respond to mechanical stimuli. Yet distinguishing which behaviors are key signatures of such mechanical feedback has proven difficult. In *D. melanogaster* body axis elongation, a canonical example of convergent extension, non-muscle myosin II (MyoII) is recruited anisotropically to cell-cell junctions where it generates force. But how the cytoskeleton is oriented remains incompletely understood.
We show how simple, physical models with a small number of biologically meaningful parameters allow us to describe the behavior of MyoII expected if the cytoskeleton responds to mechanical signals and test our conjectures using an \emph{in-toto} characterization of MyoII. We can quantitatively predict the dynamics of both the magnitude and orientation of junctional MyoII based on mechanical cues, cell deformation rates and embryo geometry. We find excellent agreement with experimental data in both wild-type embryos and when using genetic and optogenetic perturbations. Our results show how mathematical models can rationalize morphogenetic behaviors and uncover mechanisms to be targeted by molecular investigations. We add a novel perspective on developmental biology: morphogenesis may be organized by mechanical and geometric signals without constant input of genetic patterning, allowing for robust, modular and self-organized processes.

619C *Lztr1* is a conserved regulator of Ras/MAPK activity *Giovanna Collu*, Marek Mlodzik Icahn School of Medicine at Mount Sinai

LZTR1 was recently identified in a screen of human chronic myeloid leukaemia cells aimed at discovering the genetic basis of drug resistance mechanisms. Specifically, LZTR1 was shown to regulate RAS ubiquitination and RAS/MAPK pathway activation in cell culture models. Here we demonstrate a conserved function for the fly orthologue Lztr1/CG3711 in regulating Ras activity *in vivo*. Knockdown of *CG3711* leads to Ras gain-of-function phenotypes in the wing, which can be rescued by loss of one copy of *Ras*. Further, through epistasis experiments we show that CG3711 acts in the Egrf/Ras/MAPK cascade to control wing vein patterning and eye development. Consistently with the involvement of *LZTR1* in human disorders affecting the nervous system, such as Schwannomatosis, *Drosophila CG3711* is also expressed in specific neurons and glia. We are currently investigating the function of *CG3711* in these tissues, and genetic interactions with other homologues involved in similar human pathologies.

620A Robustness of Early Pattern Formation in the *Drosophila* Visual Map *Charlotte Wit*[1,2], Melinda Kehribar[1,2], Robin Hiesinger[2] 1) equal contribution; 2) Freie Universität Berlin

The *Drosophila* visual map is a highly patterned synaptic brain region formed by photoreceptor axon terminals in the lamina. We investigated the spatiotemporal organization and the underlying molecular determinants that ensure early pattern formation of this visual map. Unit eyes (ommatidia) and photoreceptor neurons (R1-R6) differentiate in a temporal wave in the developing eye disc; axon outgrowth follows the same wave pattern through the optic stalk that connects the eye disc and the lamina. Photoreceptors bundles are organized in two ways: first, each ommatidium preserves the rotational organization of R1-2-3-4-5-6 neighboring each other (intra-bundle organization); second, R1-R6 bundles preserve their relative positions to each other through the optic stalk and into the lamina (inter-bundle organization). Both types of bundle organization require the cell adhesion molecule Sidekick to preserve the pattern originating in the eye disc. Following the well-characterized differentiation order in the eye disc (R2/5, followed by R3/4, followed by R1/6), the R2 and R5 axon arrive first in the lamina, where they form an ‹equator-blind› and initially orthogonal pattern. The adhesion G-protein coupled receptor Flamingo/Starry Night (Fmi) is strongly expressed in the

early arrivals and its protein localization preserves the equator-blind and orthogonal scaffold throughout visual map formation. Loss of fmi in R2 and R5, but not in R3,4,5,6 leads to a disruption of the early pattern. The function of fmi does not seem to require canonical planar cell polarity signaling, based on frizzled and frizzled2 loss of function studies and the absence of their localization in the lamina. Mirror-symmetry around the equator is subsequently established through the positioning of R3/4 and R1/6 on opposite sides of the R2/5 axis, which again requires Sidekick. Loss of either fmi or sidekick in several neighboring cells disrupts early patterning as described above, while loss of either protein in individual photoreceptors had no discernible effects on the overall pattern. We conclude that early visual map patterning is a multi-layered, self-organizing process that is largely robust to single-cell perturbation.

621V Piezo ensures robust tissue size regulation by balancing proliferation, cell size, anisotropy and cell death *Nilay Kumar*, Megan Levis, Mayesha Sahir Mim, Maria Unger, Jeremiah J. Zartman University of Notre Dame

Mechanisms ensuring robustness in organ size regulation are critical for proper development. In epithelial tissues, mechanosensitive Piezo channels are critical for maintaining homeostasis through regulations of cell division and apoptosis. Stretch activation of Piezo triggers cell proliferation. It has been hypothesized that calcium spiking from Piezo activates ERK signaling and induces a G2-to-M transition during cell division. However, the tissue-level mechanistic and functional roles of Piezo during development remain unknown. Here, we have combined pharmacological and genetic approaches to study Piezo's roles in regulating the development of the *Drosophila* wing imaginal disc, an excellent model organism for epithelial organ development. We investigated the combined outcomes on tissue size, shape and mechanical properties. We found that Piezo overexpression increases the relative concentration levels of cytoskeletal integrin and non-muscle myosin-II leading to increased tissue curvature. Both genetic overexpression and pharmacological activation of Piezo increased proliferation and apoptosis in the tissue. Surprisingly, calcium signaling activity was blocked when Peizo was either overexpressed or knocked down but was increased with acute pharmacological activation. We hypothesize that this loss of calcium activity is due to desensitization of the Piezo channel. Further, knockdown of Piezo increased the numbers of cell neighbors, increased cell area, and decreased cell anisotropy. Piezo overexpression slightly increased the number of cell neighbors and cell area. This highlights the critical role of Piezo in regulating overcrowding within the epithelia during organ growth. In sum, these results support Piezo as a key regulator of epithelial homeostasis through a feedback mechanism that regulates the balance of forces within the epithelium. The outcomes of this feedback loop contribute to the robustness of organ size regulation.

622V Investigating the Role of Septate Junction Proteins during Border Cell Migration *Giovanni Sabatino*[1], Haifa Alhadyian[2], Robert Ward[1] 1) Case Western Reserve University; 2) University of Kansas

Border cell migration during oogenesis is an excellent system to study aspects of cell migration including polarity, leading edge dynamics, and migratory adhesion. We previously found that knocking down any of several septate junction genes in the border cells resulted in poorly penetrant defects including delayed border cell migration and fragmentation of the border cell cluster. The low penetrance is likely due to the long lifespan of septate junction proteins, coupled with the short time frame during which border cell migration occurs. The septate junction is an analogous structure to the vertebrate tight junction, allowing for occlusion in the epithelium, but septate junction proteins have also been shown to be required for morphogenetic events independent of their role in forming an occluding junction. To gain a better understanding of the function of septate junction proteins in border cell migration we are using confocal microscopy of fixed and live egg chambers expressing RNAi against coracle and Macroglobulin-complement related in heterozygous mutant backgrounds. We are particularly interested in directionality of migration, migratory cell cohesion, and the dynamics of the cytoskeleton at the leading edge. Live microscopy should allow us to observe the actin-myosin leading edge dynamics in real time, as well as better understand if loss of axial direction causes defective border cell migration. Septate junction proteins play diverse roles in embryonic development, and so, by characterizing their effects on border cell migration, we hope to discover new information about the mechanisms of embryogenesis and collective cell migration.

623V Negative feedback regulation in *Drosophila* dorsal-ventral patterning *Allison Schloop*[1], Gregory Reeves[2] 1) NC State University, Raleigh, NC; 2) Texas A&M University, College Station, TX

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*, one such morphogen is Dorsal (Dl), a transcription factor that helps with patterning of the dorsal-ventral (DV) axis in the early embryo. The impact of Dl is further refined by gene regulatory loops that help to control the dynamics of the Dl gradient. One regulatory loop of interest is the negative feedback loop with Cactus (Cact). Cact is initially bound to Dl, sequestering it to the cytoplasm, but Toll signaling on the ventral side of the cell degrades Cact and allows Dl to enter the nucleus. There, Dl can activate target genes, one of which is Cact, suggesting that Dl may regulate its own inhibition.
Our work currently focuses on establishing a system through which Cact can be examined in live embryos. Protein expression and use during development is very rapid; the turnover of Cact happens too quickly for standard live imaging

techniques, like fluorescent protein fusions. Fluorescent proteins like GFP do not have enough time to mature and fluoresce before the associated protein is degraded. To work around this, we have utilized LlamaTags (Bothma et al. 2018) to image the dynamics of Cactus. We show that Cactus, while predominantly cytoplasmic, is also in the nucleus and shows a recoverable pattern after photobleaching. This provides initial evidence for an identifiable role of nuclear Cactus.

624V Gene Regulatory Networks in Development: Genetic Variation and Robustness of Anterior-Posterior (AP) Axis Formation in *Drosophila* *Lossie (Elle) Rooney*[1], Prasad Bandodkar[2], Cranos Williams[3], Gregory Reeves[2] 1) Graduate Genetics Program, NC State University, Raleigh, NC; 2) Artie McFerrin Department of Chemical Engineering, Texas A&M University, College Station, TX; 3) Department of Electrical & Computer Engineering, NC State University, Raleigh, NC

Body plan patterning is a critical step in embryonic development that has health and viability consequences across the life of the organism. Anterior-posterior (AP) axis formation is an early event in body plan patterning and 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. We expect to identify genomic regions (QTLs) associated with these changes in expression, which we will interrogate for potential causal elements such as enhancers of AP genes. We will discuss current imaging results using ~70 lines and QTLs under investigation.

625V *trithorax* regulates the expression of multiple *Hox* genes within the embryonic dorsal vessel and is required for heart proper and aorta specification *Adam Farmer*[1, 2, 3], Shaad Ahmad[1, 2, 3], Kristopher Schwab[1, 2, 3] 1) Department of Biology, Indiana State University, Terre Haute, IN; 2) The Rich and Robin Porter Cancer Research Center, Indiana State University, Terre Haute, IN; 3) The Center for Genomic Advocacy, Indiana State University, Terre Haute, IN

The *Drosophila melanogaster* embryonic dorsal vessel is a linear myoepithelial tube comprised of a posterior large-diameter heart proper region that intakes and anteriorly propels hemolymph through a narrow tube designated the aorta. The aorta is divided into an anterior and posterior region consisting of contractile cardiac cells (CC) derived from differing lineages. The anterior-most aorta contains Tinman (Tin)-positive CCs, while the posterior aorta and heart proper CCs are arranged in repeated 'hemisegments' composed of two anterior Seven-up (Svp)-positive cells and four Tin-positive cells. Several Hox genes have critical roles in the anterior-posterior patterning of the dorsal vessel. Furthermore, the *Antennapedia (Antp)* and the *Bithorax Complex (Bx-C)* genes are expressed in a spatial colinear manner within the dorsal vessel.

In the developing embryo, *Hox* gene expression is positively regulated by *trithorax group (TrG)* genes coordinating the proper segmental and morphological identities. Here, we identify the *trithorax (trx)* gene as an essential regulator of *Hox* gene expression within the dorsal vessel. Within *trx* null embryos, the heart proper region of the stage 16 dorsal vessel remains structurally identical to the narrow posterior aorta; the posterior region does not develop the luminal dilation, nor the CCs assume the characteristic cellular morphology of heart CCs. Additionally, the posterior region of the *trx* null dorsal vessel adopts a posterior aortic fate as indicated by the loss of the heart luminal marker Multiplexin, an extracellular ligand necessary for heart luminal dilation. The homeotic transformation of the heart proper into the posterior aorta implies dysregulation of Hox gene expression with the *trx* null embryo. Indeed, Abdominal-A (Abd-A) expression is completely lost within the *trx* null dorsal vessel resembling the *abd-A* null phenotype. Additionally, other Hox genes are dysregulated, notably at the anterior and posterior ends of the dorsal vessel. The anterior-most expression of Antp was absent from the posterior aorta, while Abdominal-B (Abd-B) expression localized to the posterior-most CCs of A8 segment was also lost. Although the higher Ubx levels expressed in the posterior aorta were reduced, low levels of Ultrabithorax (Ubx) expression persisted within the dorsal vessel. Overall, *trx* is required for Antp and Bx-C protein expression within the dorsal vessel which establishes heart and aorta specification. The inactivation of *trx* results with a striking homeotic transformation of the heart proper region into the posterior aorta similar to the *abd-A* null phenotype.

626V In vivo analysis of a Hox gene enhancer required for segment-specific sense organ patterning *Xinyuan Liu*, Teresa Orenic University of Illinois at Chicago, UIC, Chicago, IL

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 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*) 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. In addition, *ScrE* is responsive to repression by Bab1/2 TFs 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 how Hox gene integration of intra-segmental patterning information leads to the development of segment-specific limb morphologies.

627V Physical mechanisms of tissue compartmentalization in the *Drosophila* embryo *Gonca Erdemci-Tandogan*, Jessica Yu, Negar Balaghi, Veronica Castle, Rodrigo Fernandez-Gonzalez Institute of Biomedical Engineering, University of Toronto, Toronto, ON

Compartment boundaries prevent cell mixing and are essential for embryonic development. Cables formed by actin and the molecular motor myosin II are often found at compartment boundaries. How boundaries are established and maintained remains unclear. In the *Drosophila* embryo, the mesectoderm separates ectoderm and mesoderm, forming the ventral midline. Eventually, mesectoderm cells are internalized becoming part of the central nervous system. We found that ectoderm and mesectoderm remained separated as the mesectoderm was internalized, suggesting the presence of a boundary between the tissues. Using live microscopy, we found an enrichment of myosin at the mesectoderm-ectoderm boundary (MEB), forming a supracellular cable. Myosin levels at the MEB decreased as the mesectoderm was internalized. To study the role of myosin cables at the MEB, we simulated mesectoderm internalization using a vertex model. Our model predicted that tension at the MEB maintains the linearity of the interface, prevents cell mixing, and controls the timing of mesectoderm internalization. Consistent with this, pharmacological inhibition of myosin disrupted the MEB, leading to mesectoderm-ectoderm cell mixing and premature mesectoderm internalization. Our model also predicted that cell divisions in the ectoderm play a role in maintaining the linearity of the MEB, a hypothesis that we are testing.

628V Identifying Proteins that Mediate Increased Proliferation at Higher Intracellular pH *Laura Martins*, Jenna Hunter, Daniel Orozco, Bree Grillo-Hill San Jose State University

Proliferation is a key cellular process that is tightly regulated in cells and essential for the 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 a 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 enhanced the *GMR>DNhe2* rough eye phenotype, *Df (2L)ED1203* and *Df(2L)ED1315* spanning 38B4-38C6. We obtained smaller deficiencies and single-gene mutations within this region to map the interaction to a single gene, and are currently testing for genetic interactions with *GMR>DNhe2*. Next, we will quantify the effects of candidate genes by quantifying proliferating cells in the third larval instar eye and wing 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.

629V Characterization of *kayak (kay)* mutant phenotypes in *Drosophila melanogaster* eye development *Manuel Alejandro Zúniga-García*[1,2], Juan Riesgo-Escovar[2] 1) Maestría en Ciencias (Neurobiología); 2) Instituto de Neurobiología (INB), Universidad Nacional Autónoma de México, Campus Juriquilla.

During development, *kayak (kay)* is a pleiotropic gene transcribed in many places and stages. We characterized the eye phenotypes of three kay alleles. We generated mutant clones for *kay*^{2}, *kay*^{3}, and *kay*^{4} using the FLP/FRT system,

and analyzed them by optical and scanning electron microscopy (SEM). Mutant *kay*^{2} and *kay*^{4} clones exhibit bristle defects by SEM. *kay*^{3} mutant clones show ommatidium and bristle defects by SEM, and sport big cuticule indentations in the medial-anterior eye region. Semithin optical sections analysis revealed ommatidium polarity and photoreceptor formation defects (ommatidiums with fewer photoreceptors). Taken together, these results indicate that kay plays an important role in eye development.

630V Quantitative input-output mapping of cytoskeleton regulator localization demonstrates linearity in developing epithelia systems *Akanksha Sachan*[1,2], Nilay Kumar[1], Alexander Dowling[1], Jeremiah J. Zartman[1] 1) University of Notre Dame; 2) IIT Bombay

The signalling pathways that operate in Drosophila are conserved with remarkable fidelity in all multicellular organisms and help us understand the emergence of birth defects and cancer in humans. For example, epithelial morphogenesis relies on coordination between multiple conserved cytoskeletal effectors; however, quantitative regulatory relationships governing cell mechanics and the tissue scale remain largely unknown. Here, we utilized immunohistochemistry, a semi-automated Python feature extraction workflow, and regression analysis to identify constitutive relationships between cytoskeletal regulators of cell mechanics in wildtype and mutant Drosophila wing imaginal discs. We evaluated possible correlation model functions between cytoskeletal regulators. In particular, we tested for linear regulation of actin accumulation by the strength of integrin concentration levels, which in turn regulates the basal curvature in the developing wing disc. RNAi-based knockdown of integrin with the Gal4/UAS system reduced basal actin levels, confirming integrin regulates actin. Both actin and integrins localize more in areas with increased local basal curvature and reduced cell height. Non-muscle Myosin II concentration also correlates with Integrin and actin concentration levels at the basal surface. Interestingly, basal actin concentration does not depend strongly on the spatial location along the Anterior-Posterior axis. Multiple polynomial relationships were analysed for predicting actin concentration, and a linear model with a single input of Integrin (or Myosin) as an independent predictor variable was selected based on the Akaike information criterion. Prediction error of the model was observed across different locations of the disc, to check how the model performs spatially. The standardized residual error was observed to be higher across the edges of the pouch region. In summary, concentrations of cytoskeletal effectors show a simple linear relationship, a signature feature of robustness in complex systems. Identification of highly collinear dependencies in biological systems also guides in constraining and calibrating biophysical models of epithelial growth and morphogenesis.

631V Investigating if the linker phosphorylation sites in Drosophila Smad2 control its stability and transcriptional activity *Edward Eivers*, Pablo Flota, Kenny Castro California State University Los Angeles

Signals from the transforming growth factor beta (TGF-β) superfamily of ligands has been shown to be important for cell fate determination, proliferation and differentiation during embryonic development and tissue homeostasis in the adult. This broad family of signaling molecules is typically divided into the bone morphogenetic protein (BMP) and Activin/TGF-β sub-families. Our labs interest, focuses on the activities of a group of TGF- β proteins known as receptor Smads (R-Smads). These transcription factors transmit intracellular signals for each pathway when phosphorylated in their C-terminal domains by ligand-activated transmembrane receptors. In this study, we identified four potential phosphorylation sites in the central linker domain of Drosophila Smad2 (dSmad2). We present data showing that mutation of these linker sites into non-phosphorylatable alanines resulted in stabilization of C-terminally phosphorylated dSmad2 proteins. We also found that when dSmad2 linker mutants were misexpressed in Drosophila wing imaginal discs using Gal4 drivers, adult wings were, reduced in size, bifurcated and displayed severely disrupted venation when compared to control wings. We are also investigating if mutation of the linker phosphorylation sites in dSmad2 can affect expression of downstream TGF- β target genes. In conclusion, our experiments aim to broaden our understanding of the signaling activities of the TGF-β pathway, in particular dSmad2 signal regulation and duration during Drosophila wing development.

632V Frizzled receptor-mediated mechanisms of Wingless signaling in developing *Drosophila* wing epithelium *Swapnil Hingole*, Varun Chaudhary Indian Institute of Science Education and Research (IISER) Bhopal, India

Wnts are evolutionarily conserved lipid-modified glycoproteins. Secreted Wnts can travel up to several cell distances over receiving cells and generate a response in a concentration-dependent manner. Their ability to regulate the growth and differentially pattern the tissue makes them crucial for embryonic development and adult tissue homeostasis. Wnts can act directly at a short-range and long-range, which is best studied in the developing *Drosophila* wing imaginal disc. Wingless (Wg, Wnt1 homolog in *Drosophila*) is secreted from narrow stripes of cells along the dorsoventral boundary of the wing disc and activates both short and long-range signaling. However, the mechanisms of long-range gradient signaling are sparsely understood and highly debated. Replacing endogenous Wg with membrane-tethered Wg was able to maintain long-range target gene expression even in the absence of gradient in wing disc and developed a normally patterned wing. Moreover, studies have also demonstrated that long-range target gene expression can be maintained in a ligand-independent manner.
Our findings show that Frizzled 2 (Fz2), a receptor of Wg, maintains target gene expression beyond the reach of Wg

protein in a ligand-independent manner. Fz2 acts redundantly with Fz1 to activate Wg-dependent canonical signaling. However, we found that the maintenance of signaling is a novel non-redundant function of Fz2. This makes Fz2 highly important for the survival and patterning of cells in the absence of the proper Wg gradient. We also show that the Fz2 mediated maintenance of signaling is not dependent on other Wnt ligands. Together these findings and the elevated Fz2 levels in the absence of Wg protein suggest that receptor oligomerization and internalization could be possible mechanisms behind the maintenance of signaling. Thus the combinatorial effect of direct Wg dependent signaling and Fz2 mediated ligand-independent maintenance provides robustness to the developing wing epithelium experiencing varying concentrations of Wg ligand.

633V Modulation of integrin levels triggers actomyosin reorganization essential for proper tissue folding *Andrea Valencia Expósito*[1], Nargess Khalilgharibi[2], Yanlan Mao[2], María Dolores Matín Bermudo[1] 1) Centro Andaluz de Biología del Desarrollo, CSIC/Universidad Pablo de Olavide/JA, Sevilla, Spain; 2) MRC Laboratory for Molecular Cell Biology, University College London, London, UK

Folding of epithelial tissues is essential for the formation of complex three-dimensional organ structures during development. However, while folding of epithelial tissues towards the apical surface has been long studied, little is known about the mechanisms underlying epithelial folding towards the basal side. Both apical and basal epithelial tissue folding are accompanied by changes in actomyosin organization and in cell adhesion. Manipulation of apical adhesion molecules, such as cadherins, affects actomyosin dynamics and apical folding. Similarly, adhesion of the cells to the basement membrane (BM), via integrins, is required for proper basal epithelia folding. Nevertheless, how changes in cell adhesion and actomyosin organization interact with each other to drive epithelial folding during morphogenesis remains unknown. Here, we use the primordium of the wing, the *Drosophila* wing imaginal disc epithelium, as a model system to analyse the role of integrins on the regulation of the actomyosin rearrangements and cell shape changes underlying basal tissue folding. The wing imaginal disc is an epithelial sac contacting on its basal side with a BM. During its development, the disc folds basally along a row of cells, the future wing margin cells. In this work, we show that basal disc folding involves four interconnected events: reduction on integrin levels, increase of F-actin accumulation basolaterally, cell shortening and detachment from the BM. We show that ectopic maintenance of high levels of integrins in wing margin cells prevents these changes, leading to abnormal folding of the epithelium. Contrariwise, reduction of integrin levels in an ectopic location in the disc recapitulates the events that take place at the wing margin, resulting in the formation of an ectopic fold. Finally, through computational exploration, we have found that the reduction of adhesion to the BM must preceed the changes in F-actin reorganization and cell shortening to allow proper folding of the epithelium. Based on these results, we propose that changes in the adhesion of cells to the BM mediated by integrins trigger modifications in the actomyosin network necessary for basal epithelial folding during organogenesis.

634V Apterous Regulates the Formation of Stable Myotendinous Junctions in the Drosophila Embryo Basya Buchbinder[1], Xyomara Wutoh-Hughes[1], Mary Baylies[3,4], *Krista Dobi*[1, 2] 1) Department of Natural Sciences, Bernard M. Baruch College, City University of New York, New York, NY; 2) PhD Program in Biology, The Graduate Center, City University of New York, New York, NY; 3) Developmental Biology Program, Sloan Kettering Institute, Memorial Sloan Kettering Cancer Center, New York, NY; 4) Biochemistry, Cell & Developmental Biology, and Molecular Biology (BCMB) Program, Weill Cornell Graduate School of Medical Sciences, New York, NY

Skeletal muscles are required for locomotion such as walking, grasping, blinking and chewing. To accomplish these movements, muscles must form secure tendon attachments that can withstand the force of contraction. It is vital that strong attachments form between the right pairs muscle and tendons during development. These myotendinous junctions link transmembrane Integrin proteins to the cytoskeleton and sarcomere. In the Drosophila embryo thirty distinct muscles make up each abdominal hemisegment. While certain muscle subsets are known to have specific guidance cues (for example, ventrolateral muscles use the protein Kon-tiki), a gap in our knowledge exists about how the lateral transverse muscles regulate where and how their attachments are formed. We have identified the transcription factor Apterous as important for the formation of robust muscle attachments. Apterous is expressed in and required for the appropriate patterning of all four lateral transverse muscles in each abdominal hemisegment. We find that embryos with gain or loss of Apterous function display attachment defects, including missing or incorrect attachments. Misexpression of Apterous leads to severe disruptions to the muscle pattern, including an increase in lateral transverse-like muscle morphologies. Moreover, using time-lapse confocal imaging, we show that misexpression of *apterous* in the somatic musculature leads to loss of muscle attachment upon the onset of contractions, resulting in an inability to hatch and embryonic death. We demonstrate that overexpression of *apterous* leads to loss of both beta-PS Integrin and alpha-PS2 Integrin from myotendinous junctions. Our work establishes a clear function for *apterous* in the regulation of muscle attachment, linking changes in gene expression to alterations in muscle morphology.

635V The Thanos Requirement for Transdetermination Leads to an End Game on Wing Cell Fate as Ectopic Eyes Develop *Alison Smith*, Justin Kumar Indiana University Bloomington

As development begins cells are in a pluripotent state, but via cell signaling pathways and morphogen concentration

gradients cells begin to determine towards their final cell fate. Various developmental anomalies arise when a cell does not adopt or retain its correct fate. A process where a determined cell switches its fate to that of another without dedifferentiation is via transdetermination. Transdetermination can be studied in *Drosophila* via ectopic eye formation. Ectopic eyes can be formed through the misexpression of the master regulator gene of the retinal determination network *eyeless* (*ey*). Though it is more complex than a wing imaginal disc's cell immediately switching its fate to that of a retinal fate solely with the initiation of *ey* misexpression. There are also certain requirements that must be met - the Thanos requirement. The Thanos requirement has five pillars including: spatial misexpression, co-expression of Dpp, magnitude of misexpression, initiation and duration of *ey* misexpression. I hypothesize that during this transdetermination as the cells move from their wing fate to that of retinal fate the cells proceed through a biphasic state where the cell is not solely fated as either wing or retinal. This is hypothesized due to my work using the UAS-GAL4 misexpression system. With this system I uncovered a previously undiscovered pillar of the Thanos requirement: duration of *ey* misexpression. This uninvestigated requirement is as follows, for an ectopic eye to form there must be GAL4 expression to drive *ey*misexpression not only in the wing disc but also the in the eye field of the eye antennal disc. This is such that as the cells begin to take on a more retinal fate than wing if the GAL4 driver is active in only the wing imaginal disc it will turn off, ceasing *ey* misexpression and no ectopic eye will form. Though if the GAL4 driver is active in both the wing imaginal disc and the eye antennal disc even as the cell takes on a more retinal fate the GAL4 enhancer will remain on, *ey* misexpression will continue, and an ectopic eye will form (if the four other pillars are also met). In the cases where all of the five pillars of the Thanos requirement are met there is an end game on wing fate as the cells adopt their new retinal state. I am further investigating the Thanos requirement using immunofluorescence and quantitative PCR. By understanding the requirement of transdetermination we may better understand the mechanism of transdetermination. By better understanding the mechanism of transdetermination we can not only gain a better understanding of cell fate determination, but what goes awry when cells switch their fates during development.

636V The JNK and Hippo pathways regulate an overlapping transcriptome to control neoplastic tissue growth *Katrina Mitchell*[1,2], Joseph Vissers[4], Kieran Harvey[1,2,3] 1) Peter MacCallum Cancer Centre, Melbourne, Australia; 2) Sir Peter MacCallum Department of Oncology, University of Melbourne, Australia; 3) Depart of Anatomy and Developmental Biology, Monash University, Australia; 4) Department of Clinical Pathology, University of Melbourne, Australia

The final size of an organ is determined by both organ-extrinsic and organ-intrinsic factors, which can adapt to changes in environmental conditions and tissue damage. Homeostatic mechanisms eliminate damaged or abnormal cells to prevent tumour growth, which in turn facilitates compensatory proliferation to maintain organ size. In growing *Drosophila melanogaster* epithelial tissues, cells with disrupted apical basal polarity are eliminated by neighbouring wild type cells through cell competition. The JNK pathway limits the growth and viability of neoplastic cells and has been reported to do this by suppressing the key Hippo pathway transcriptional regulatory protein Yorkie. Using targeted DamID and transcriptomics, we have identified target genes of the JNK and Hippo pathway transcription factors and found that these pathways act in parallel to regulate overlapping target genes. Yorkie activates the transcription of genes that promote cell proliferation and survival, whilst the JNK pathway transcription factors Jun and Fos repress transcription of these genes specifically in neoplastic cells. Additionally, we have identified a co-repressor that Jun/Fos cooperates with to prevent the uncontrolled growth of neoplastic tumours. These findings shed new light on organ size control, tissue damage and tumour suppression.

637V The Osiris family genes regulate endocytic trafficking during Drosophila tracheal maturation *Lan Jiang*, Aaron Scholl, Istri Ndoja, Doria Morante, Abigail Ivan Oakland Univeristy

The *Drosophila* trachea is a ramifying network of epithelial tubes with a monolayer of epithelial cells surrounding an apical lumen. Following the formation of continuous tubes, the tube maturation process follows. It is a multistep process: (a) apical secretion to form an apical lumen containing chitin-based extracellular matrix, during which tubes expand in both diameter and length, (b) solute clearance to remove solid luminal matrix and liquid in the lumen, and (c) air filling to inflate the entire trachea. These changes occur at the apical side of the tracheal tubes with little or no changes to the basolateral side.
The *Osiris (Osi)* gene family is located at a locus that exhibits dosage sensitivity (triplo-lethal and haplo-lethal). The protein sequence annotation of Osi proteins identified an endo/lysosomal signal, a transmembrane domain, a domain of unidentified function DUF1676, and an AQXLAY motif, suggesting that these proteins are likely involved in vesicular trafficking. However, the functional analyses of *Osi* genes are very limited. Only a few studies indicate the potential role of *Osi* genes in endosome-mediated protein trafficking. For example, RNAi knockdown of *Osi 21* suggests its involvement in lysosome-mediated degradation of endocytosed rhodopsin in *Drosophila* eye. In addition, *Osi23* is essential for the formation of nanopores lining the olfactory sensillum in *Drosophila,* potentially through endo/lysosome-mediated trafficking. However, the role of *Osi* genes in *Drosophila* trachea is still unknown.
Previously we identified several *Osi* genes with obvious expression in the *Drosophila* trachea from stage 14 until the end of embryogenesis. In addition, expressing HA-tagged Osi proteins in trachea revealed their localization in vesicle-like

structures, suggesting their potential roles in vesicular trafficking. Immunohistochemistry using Osi specific antibodies further confirmed that the endogenous Osi proteins are localized at various endosomes in tracheal cells.
The potential functional redundancy of *Osi* genes prompted us to generate *Osi* single, double, and triple loss-of-function mutants using CRISPR-cas9. No obvious phenotypes were observed in single mutants. However double and triple mutants showed strong defects in trachea tube maturation. Genetic interaction studies suggest that *Osi* genes are required for trachea tube maturation through endosome-mediated protein trafficking.

638V The adult *Drosophila* salivary gland exhibits an unusual mode of cell division Caitlin van Ree, Nicole Dominado, Nicole Siddall, *Gary Hime* Department of Anatomy and Physiology, University of Melbourne, Parkville, VIC , Australia

Larval salivary glands of *Drosophila* are well known to exhibit polytene salivary glands formed via endoreplication and have been long used to study this process. In contrast, less 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. The epithelial cells are polyploid and larger than the imaginal ring cells from which they derive. The adult salivary epithelial cells do not undergo mitosis as they do not express phosphorylated Histone H3, 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 of the cuboidal epithelium 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 may be an unusual mechanism to be utilised in primary formation of a tissue but it may be the only efficent way for polyploid cells to increase in number. The adult *Drosophila* salivary gland will serve as a model for genetic analysis of this mode of division.

639V Cling film – a novel regulator of epithelial morphogenesis *Clara-Maria Ell*[1,2,3], George Pyrowolakis[2,3] 1) Spemann Graduate School of Biology and Medicine (SGBM), Albert-Ludwigs University of Freiburg, Germany; 2) CIBSS - Centre for Integrative Biological Signalling Studies, Albert-Ludwigs University of Freiburg, Germany; 3) Faculty of Biology, Institute for Biology I, Albert-Ludwigs University of Freiburg, Germany

Epithelial morphogenesis is essential for the transformation of simple epithelial sheets into complex organs.
The *Drosophila* larval wing undergoes drastic changes during pupal development and has served as a valuable model to address the underlying mechanisms at tissue-, cell- and subcellular-level. Changes include proliferation, cell rearrangements, cell shape modulation and drastic extracellular matrix (ECM) remodeling, all of which are tightly regulated by intrinsic and extrinsic cues and forces. Here we describe *Cling film* (*Cling*), a target of patterning cues in larval wing discs, as a novel regulator of wing morphogenesis. *Cling* encodes a transmembrane protein with a large, multi-domain extracellular region which predominantly localizes to the apical cell surface. Expression of *cling* starts in a spatially defined pattern in late larval wing discs and becomes more uniform in the early pupal stages. Adult wings of *cling* mutants are severely malformed and strongly folded. We could show that defects in *cling* mutants manifest in early pupal stages and that *cling* mutant pupal wings increase their surface normally but fail to stretch out along the proximo-distal axis resulting in heavily folded wings. The phenotype is accompanied by failure to degrade the apical ECM, while genetic ablation of the apical ECM component Dumpy can fully restore wing expansion in *cling* mutants. Our results indicate that Cling is critically involved in patterned ECM remodeling during early wing morphogenesis. We are currently investigating interactions of Cling with structural ECM components and the ECM degrading proteases Stubble and Notopleural.

640V *dysfusion* negatively regulates JAK/STAT signaling to constraint the invasive cell population *Jhen-Wei WU*[1], Chueh-Wen Wang[1], Ruo-Yu Chen[1], Liang-Yi Hung[1], Yu-Chen Tsai[3], Yu-Ting Chan[1], Yu-Chiuan Chang[2], Anna C-C Jang[1] 1) Department of Biotechnology and Bioindustry Sciences National Cheng Kung University, 1 University Rd, Tainan City 70101, Taiwan; 2) Institute of Biomedical Sciences, National Sun Yat-sen University, 70 Lien-Hai Rd, Kaohsiung 804, Taiwan; 3) Department of Life Science and Life Science Center, Tunghai University, No.1727, Sec.4, Taiwan Boulevard, Taichung City 407224, Taiwan

Cell migration is a critical process for embryonic development and cancer metastasis. How the epithelial cells are selected and adapt migratory cell fate remains unclear. To decipher the underlying mechanism, we apply border cells (BCs), a small group of cells disseminating from the epithelium and migrating through germ cells *Drosophila* oogenesis, as a model to study collective cell movement. In a forward genetic screen, we found that overexpression of *dysfusion* (*dysf*) which severely impeded BC migration. Functional analysis further shows that depletion of *dysf* in BCs completely blocked cell mobility but that in polar cells, a specialized pair of cells secreting Upd to transactivate JAK/STAT signaling in the neighboring cells to form a cluster, leads to 64% of BC migration delay. These results indicate the

requirement of Dysf in both border and polar cells during migration. Interestingly, overexpression of UAS-*dysf* in BCs hampered the recruitment, only 3.2 BC observed in the cohort, in comparison to 6 BC in the wild type. To teste whether the reduction of BC number result from JAK/STAT signaling hypoactivation, we examined STAT activity by STAT-GFP reporter under *dysf* overexpression. In wild type BCs, the intensity of STAT-GFP gradually increased during migration, but ectopically upregulated in the *dysf* mutant cells, and extra border cells were also observed simultaneously. Consistently, the ectopic BC phenotype caused by JAK or Upd overexpression can be suppressed by overexpression of UAS-*dysf*. Interestingly, Dysf protein is resided at the nuclear membrane of all germline and follicle cells but specifically reduced in BCs upon migration. When BCs reach the destiny, oocyte, their Dysf becomes undetectable. Moreover, we observed that nuclear/cytosol (N/C) ratio of STAT was greatly reduced up overexpression of *dysf* in the salivary gland. To elucidate how Dysf regulatess STAT nuclear transport, we carried out a biochemical screen to seek for interacting proteins with endogenous knock-in tagged Dysf. We found that Pendulin, a member of the Importin-alpha protein family, interact with Dysf. Therefore, we propose that *dysf* may determine the size of border cell clusters by regulating JAK/STAT signaling.

641V Characterization of adhesion and secretin GPCRs in the salivary glands and germ cells during *Drosophila* embryogenesis *Sean Riccard*, Caitlin Hanlon Quinnipiac University

Embryogenesis requires coordinated cell migration directed by molecular guidance cues for proper tissue development. G-protein coupled receptors (GPCRs) are widely conserved transmembrane receptors that relay extracellular signals to the intracellular environment. Despite their prominence, GPCRs are understudied during *Drosophila* embryonic development. The adhesion and secretin GPCR subfamilies, characterized by their long N-terminal domains, are an interesting topic for further study because of their role in other developmental processes including cell adhesion and endocrine signaling. Adhesion GPCRs are highly expressed in mouse embryonic kidney structures, promote angiogenesis in cell culture, and are implicated in human skeletal development and disease. Secretin GPCRs are also implicated in human skeletal development as well as retinal angiogenesis in mice. Here, we used RNAi to tissue-specifically knockdown the adhesion and secretin GPCRs to determine their role in the embryonic salivary glands (SG) and germ cells (GC). We found that adhesion GPCRs influence *Drosophila* SG structure in the late embryo. Knockdown of *CG11318* or *CG15556* has resulted in irregular SG placement ($p<0.05$). Furthermore, Crumbs-stained SGs in embryos with a knockdown for *CG15556* have shown that the apical domain of these SGs are uneven, not smooth and straight like the wild type SG. Interestingly, knocking down either of these gene types in the GCs has had no effect on GC migration. These findings suggest a role for adhesion GPCRs in proper embryonic SG migration. Our next steps are to observe SG migration in embryos with deficiency and insertion lines that cover *CG11318*, as well as characterize embryonic expression of *CG11318* by *in situ* hybridization.

642V Dunk Regulates Cortical Localization of Myosin II during *Drosophila* Cellularization through Interaction with the Scaffolding Protein Anillin *Jiayang Chen*, Bing He, Melissa Wang Dartmouth College

Cleavage is a common step of early embryonic development, generating a monolayer of epithelial cells at the surface of the embryo called “blastoderm”. In *Drosophila*, this process is achieved by cellularization, a special form of cytokinesis. Similar to typical cytokinesis, cellularization is initiated by recruiting non-muscle myosin II (“myosin”) to the cleavage furrows. Myosin first forms an interconnected hexagonal array at the base of the invaginating cleavage furrows and subsequently reorganizes into individual contractile rings. We have previously identified a gene *dunk* that promotes myosin retention at the basal array during early cellularization, but the underlying mechanism is unclear. In this work, we performed a genome-wide yeast two-hybrid screen and identified anillin (Scraps in *Drosophila*), a conserved cytokinesis scaffolding protein, as the primary binding partner of Dunk. Anillin has been reported to regulate the formation of cytokinetic rings during cellularization, but it is unclear whether it regulates the basal myosin array before ring formation. We found that anillin extensively colocalized with the basal myosin array during early cellularization. This colocalization could be detected as early as the onset of cellularization when myosin was first recruited to the cortex as discrete puncta. During the formation of nascent cleavage furrows, anillin and myosin remained colocalized in the cortical puncta as they moved to the cleavage furrows. In *anillin* mutant, myosin showed a biased localization to the vertices of the basal array and was depleted from edges, closely resembling the myosin phenotype in *dunk* mutant embryos. Dunk and anillin also display functional interactions. In *dunk* mutant embryos, the localization of anillin at the basal array was severely disrupted. Furthermore, embryos doubly heterozygous for *anillin* and *dunk* showed synthetic defects during cellularization. Finally, *dunk* and *anillin* mutants showed similar synthetic phenotypes when combined with mutations in Bottleneck, an actin-bundling protein functioning to restrain actomyosin contractility during cellularization. Together, our results suggest that Dunk regulates myosin at the basal array by interacting with anillin and regulating its cortical localization. Our work demonstrates a previously unappreciated role for anillin in regulating cortical myosin dynamics in early cellularization and may shed light on the regulation of myosin in other cytokinetic processes.

643V Mechanical bistability of the mesoderm facilitates mesoderm invagination during *Drosophila* gastrulation *Hanqing Guo*[1], Michael Swan[2], Bing He[1] 1) Dartmouth College; 2) Princeton University

Apical constriction driven by non-muscle myosin II (“myosin”) provides a well-conserved mechanism to mediate

epithelial folding. It remains unclear how contractile forces near the apical surface of a cell sheet drive out-of-plane bending of the sheet and whether myosin contractility is required throughout folding. We developed a CRY2-CIB based optogenetic system ("Opto-Rho1DN") to inhibit the myosin activator Rho1 during *Drosophila* mesoderm invagination, a typical epithelial folding process mediated by apical constriction. Stimulation of Opto-Rho1DN resulted in rapid myosin dissociation from the cell cortex and disassembly of apical actin, causing acute loss of actomyosin contractility. Interestingly, we find that inhibition of actomyosin contractility during the early, "priming" stage of invagination causes reversal of invagination (the "Early group"). In contrast, invagination continues when inhibition is imposed during the actual folding step after the tissue passes through a stereotyped transitional configuration (the "Late group"). This binary response to actomyosin inhibition suggests that the mesoderm is mechanically bistable during gastrulation. Through 3D reconstitution, we show that apical relaxation happens in both groups after stimulation but has distinct impact on cells that have bent towards the ventral midline due to apical constriction. In the Early group, the bent cells unbend. In the Late group, however, the bent cells keep bending towards the ventral midline as invagination continues, suggesting the presence of additional mechanical input other than actomyosin contractility. Computer modeling suggests that the observed mechanical bistability can arise from an in-plane compression from the surrounding ectoderm, analogous to a buckling process. In line with this model, we find that the transitional state coincides with an apical-basal cell shortening in ectoderm, which may generate in-plane compression through cell volume conservation. Importantly, ectodermal shortening still occurs in the absence of apical constriction, indicating that they can provide distinct mechanical input for mesoderm folding. Taken together, our results demonstrate mechanical bistability in *Drosophila* mesoderm during gastrulation and suggest that mesoderm invagination is mediated by a joint action of local apical contractility and global tissue compression to trigger epithelial buckling.

644V Shaping 3D geometry in tubulogenesis: a PDZ domain-containing protein Arc regulates Crumbs to determine salivary gland morphology in Drosophila embryogenesis *Ji Hoon Kim*, Kwon Kim, Devin Vertrees, Rika Maruyama, Deborah Andrew Johns Hopkins University School of Medicine, Baltimore, MD

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. Importantly, Arc co-localized 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.

645V Cell polarity determinant Dlg1 regulates the spatial organization and contractile behavior of non-muscle myosin II during tissue morphogenesis Melisa Fuentes, *Bing He* Dartmouth College

Contractile forces generated by non-muscle myosin II ("myosin") are critically involved in many morphogenetic processes in animal development. In this work, we found that the cell polarity determinant Dlg1 regulates the spatial distribution and contractile behavior of myosin during tissue folding and tissue elongation in early *Drosophila* embryos. During *Drosophila* gastrulation, ventrally localized mesodermal cells undergo apical constriction and invaginate to form a ventral furrow. We found that depletion of Dlg1 disrupts the transition between apical constriction and invagination without affecting the rate of apical constriction, the main driving force for furrow formation. In Dlg1 deficient embryos, cells adjacent to the constriction domain ("flanking cells") display a reduced level of apical adherens junctions and ineffective apical myosin contractions. These defects result in overstretching of the apical domain of the flanking cells and weakening of mechanical coupling between the mesoderm and the neighboring ectoderm, which we show is sufficient to cause delay in invagination. After ventral furrow formation, the germband epithelium doubles in length along the anterior-posterior axis by shortening along the dorsal-ventral axis, a process mediated by cell intercalation. During cell intercalation, planar polarized localization of myosin facilitates patterned shrinking of anterior-posterior

junctions and extension of dorsal-ventral junctions. We found that loss of Dlg1 in the germband results in a decrease in junctional myosin, an increase in medio-apical myosin, and ectopic accumulation of myosin along the lateral membrane. Further mutant analysis demonstrates that the SH3 and GUK domains of Dlg1 are both important for the proper spatial organization of myosin during germband extension. The myosin regulator Rho-associated kinase (Rok) displays a similar mis-localization pattern as myosin upon loss of Dlg1. These defects are accompanied by ectopic apical constriction in the germband and a reduced rate of tissue extension. Together, our findings reveal the function of Dlg1 in regulating subcellular distribution and/or contractile behavior of myosin in multiple tissues under various developmental contexts.

646V The role of Scabrous in long distance Notch signaling during bristle patterning *Adam Presser*, Ginger Hunter Clarkson University

Correctly ordered thoracic bristles are necessary for the fly to effectively sense its environment. Notch signaling is critical for the patterning of these bristles: cells in the developing notum epithelium self-organize into Delta-expressing bristle precursor cells and Notch-activated epithelial cells. In order to maintain wildtype bristle spacing, long-distance signaling is necessary. This can occur via either filopodia-mediated Notch signaling or paracrine signaling by diffusible Notch regulators. Scabrous is a secreted protein expressed in bristle precursor cells, and the dynamics of Scabrous signaling in live notum are under-characterized. Based on the literature, we hypothesized that Scabrous acts as a positive regulator of Notch signaling at a distance. Furthermore that Scabrous activity is independent of filopodia-mediated, long-range Notch signaling. Here, we describe several aspects of Scabrous signaling including the location of Scabrous within the patterning tissue using live confocal imaging of Drosophila pupae and fluorescence-stained, dissected pupal nota. We also analyze Notch signaling in response to changes in Scabrous expression levels. These data will help to improve current models of tissue patterning driven by Notch-mediated lateral inhibition.

647V How to form and maintain a monolayered epithelium: the role of integrins *Lourdes Rincón-Ortega*, Acaimo González-Reyes, María Dolores Martín-Bermudo Centro Andaluz de Biología del Desarrollo, CSIC-Univ. Pablo de Olavide, Sevilla, Spain

Integrins are essential proteins that connect the cell with the extracellular matrix (ECM) and have an important role in the development and maintenance of epithelia. They are implicated in many epithelial diseases and also in tumor progression. To study integrin function in epithelial homeostasis, we use the follicular epithelium (FE) of the *Drosophila* ovary as model system.
The monolayered FE present, in the developing egg chambers of the ovary, follicle cells (FCC), the most abundant cell type, and polar cells, that act as signaling centers for the patterning of the FE. Individual egg chambers are connected by a line of stalk cells. The FE is surrounded by a specialized ECM known as basement membrane (BM), which supports egg chambers growth during oogenesis. The FE is connected to the BM through integrins. Integrins are essential to maintain the architecture of the FE, since the absence of integrins induces extra-layers of FCC at the poles of the egg chambers (Fernández-Miñán et al., 2007). In addition, we have recently observed that lack of integrins produces alterations in the number and distribution of polar and stalk cells, which results in aberrant follicles.
In this project, we aim to understand how these phenotypes arise, thus gaining insight into the role of cell-ECM interactions mediated by integrins during epithelial morphogenesis and maintenance. Our results show that integrins control the number of polar and stalk cells by regulating the proliferation rate and activation of signaling pathways in their precursors. Furthermore, analysis of cell division *in vivo*, leads us to propose that the formation of extra-layers could be due to a combination of aberrant spindle orientation, cellular tension and BM mechanical properties. Taking all together, our findings show that integrins regulate epithelia development and maintenance by different molecular and cellular mechanisms, including activity of signaling pathways, spindle orientation and mechanical properties of cells and their surrounding BM.

648V Myosin XV regulates basal filopodia formation during bristle patterning *Rhiannon Clements*[1], Aidan Cahill[1], Luke Cowert[2], Ginger Hunter[1] 1) Clarkson University, Potsdam, NY; 2) The Pennsylvania State University, University Park, PA

The ability of Notch signaling to drive the formation of a broad range of biological patterns relies, in part, on the activity of cellular protrusions that allow contact between cells at a distance. One example of this is the patterning of sensory bristles on the thorax of Drosophila melanogaster. In this tissue, thin, dynamic, actin-based, filopodia which extend from the basal surface of the patterning epithelia and are essential for long-range Notch signaling. We have identified Myosin XV as a regulator of the formation and maintenance of basal filopodia in the notum. Myosin XV has previously been shown to localize to, and play a role in the dynamics of, filopodia in Drosophila as well as in other cellular protrusions in mammals, including stereocilia. Using a combination of genetics, cell biology, and confocal imaging, we find that Myosin XV contributes to the density of sensory bristles through its role in regulating basal filopodia dynamics. Currently we are using a CRISPR-based strategy to generate a null mutation in the myosin XV gene. We observe that Notch signaling is affected by specific targeting of basal filopodia, through the use of a transcriptional reporter of Notch signaling. Together these results support a role for basal filopodia in lateral inhibition during bristle patterning.

649V 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], Andrew Kump[1,2,3], Manoj Panta[1,2], Kristopher Schwab[1,2,3], Mark Inlow[2,4], Shaad Ahmad[1,2,3] 1) 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, Indiana State University, Terre Haute, IN; 4) Department of Mathematics and Computer Science, Indiana State University, Terre Haute, IN

Forkhead/Fox transcription factors (TFs) mediate multiple cardiogenic processes in both mammals and *Drosophila*. We showed previously that the *Drosophila* Fox genes *jumeau* (*jumu*) and *Checkpoint suppressor 1-like* (*CHES-1-like*) control three categories of cardiac progenitor cell division—asymmetric, symmetric, and cell division at an earlier stage—by regulating Polo kinase activity, and mediate the latter two categories in concert with the TF Myb. Those observations raised two questions: whether other Fox TF-controlled genes mediating cardiac progenitor cell divisions were also regulated by both *CHES-1-like* and *jumu* in a *polo*-like manner and whether such Fox-regulated genes mediated all three categories of cardiac progenitor cell division or a subset thereof. By comparing transcriptional expression profiles of wild-type, *jumu* loss-of-function, and *CHES-1-like* loss-of-function mesodermal cells, we identified multiple genes transcriptionally activated by *jumu*, but not regulated by *CHES-1-like*. Phenotypic analysis of mutations showed that two of these exclusively *jumu*-regulated targets, the kinesin-encoding gene *nebbish* (*neb*) and the anilin-encoding gene *scraps* (*scra*) are 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 *neb*, *scra*, *jumu*, and *polo*; between *neb* and *Myb*; the absence of such synergistic interactions between either *scra* and *CHES-1-like* or *neb* and *CHES-1-like*; and the rescue of solely symmetric and earlier cardiac progenitor cell division defects in *jumu* mutants by ectopic cardiac mesoderm-specific expression of *neb* demonstrate that *scra* and *neb* comprise an exclusively *jumu*-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-encoding gene *pavarotti*, the citron kinase-encoding gene *sticky*, and the Rho GTPase-encoding gene *tumbleweed* may be other components of this subnetwork. Using additional genetic interaction and rescue assays, we are attempting to position *neb* and *scra* topologically relative to each other and these other potential subnetwork components. Collectively, our results illustrate how an individual regulator can utilize different combinations of downstream effectors to control distinct developmental processes.

650V Exploring the mechanistic roles of APC in the Armadillo/β-catenin destruction complex Katherine Gerber, Julia Kiefer, Emily Errickson, Sonia Hafiz, Matthew Krause, Carmen Navia, Hannah Salvucci, *David Roberts* Franklin & Marshall College, Lancaster, PA

The tumor suppressor, Adenomatous Polyposis Coli (APC), is an important negative regulator of the Wg/Wnt signaling pathway and is inactivated in nearly 80% of all colon cancer cases. APC participates in a multi-protein "destruction complex" that phosphorylates the proto-oncogene, Armadillo/β-catenin (Arm/β-cat), thereby targeting 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, and prior research from several groups has suggested that these sites play important mechanistic roles in the destruction complex, suggesting specific models of how APC contributes to the destruction complex. We have been testing proposed models using a structure/function approach using Drosophila APC2 as a model system. 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 Arm/β-cat binding sites on APC and Axin could be redundant in cells that do not receive Wg/Wnt signaling, but that APC's Arm/β-cat binding sites may play a unique mechanistic role(s) in cells that receive Wg/Wnt signal. To test the redundancy hypothesis, we have generated APC2 and Axin transgenes either containing or lacking Arm/β-cat binding sites. Additionally, to explore that possibility that APC's Arm/β-cat binding sites have a unique mechanistic role, we have generated 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 are required for efficient Arm/β-cat destruction.

651V *C. elegans* Notch proteins are tuned to lower force thresholds than *Drosophila* Notch, bypassing the requirement for Epsin-mediated ligand endocytosis. *Paul Langridge*[1,2], Alejandro Garcia-Diaz[2], Jessica Chan[2], Iva Greenwald[2], Gary Struhl[2] 1) Augusta University, Georgia, GA; 2) Columbia University, New York, NY

The conserved transmembrane receptor Notch has diverse and profound roles in controlling cell fate during animal development. In the absence of ligand, a Negative Regulatory Region (NRR) in the Notch ectodomain adopts an autoinhibited confirmation, masking a protease cleavage site; ligand binding induces cleavage of the NRR, leading to

Notch ectodomain shedding as the initiating step of signal transduction. In *Drosophila* and vertebrates, recruitment of transmembrane protein ligands by the endocytic adaptor Epsin, and their subsequent internalization by Clathrin-mediated endocytosis, exerts a «pulling force» on Notch that is essential to expose the cleavage site in the NRR. Here, we show that Epsin-mediated endocytosis of transmembrane ligands is not required to activate the two *C. elegans* Notch proteins. Using an *in vivo* force sensing assay in *Drosophila*, we present evidence (i) that the *C. elegans* NRRs are tuned to lower force thresholds than the NRR of *Drosophila* Notch, and (ii), that this difference depends on the presence of a "Leucine plug" that occludes the cleavage site in the *Drosophila* and vertebrate Notch NRRs but is absent from the *C. elegans* Notch NRRs. Our results thus establish an unexpected evolutionary plasticity in the force-dependent mechanism of Notch activation and implicate a specific structural element, the Leucine plug, as a potential determinant.

652V Structural basis of the Calpain A:Cactus (IκB) complex reveals fit induced and competition based mechanisms that alters NFκB activity in embryonic patterning and the immune response *Alison Julio*[1], Paloma Dias e Vasconcellos[1], Priscila Gomes[2], Maira Cardoso[1], Pedro Pascutti[2], Ethan Bier[3,4], Paulo Bisch[2], Helena Araujo[1,5] 1) Instituto de Ciências Biomédicas, Universidade Federal do Rio de Janeiro, RJ. Brazil; 2) Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro, RJ. Brazil; 3) Section of Cell and Developmental Biology, University of California, San Diego, La Jolla, CA, USA; 4) Tata Institute for Genetics and Society, University of California, San Diego, La Jolla, CA, USA; 5) Instituto Nacional de Entomologia Molecular (INCT-EM), Universidade Federal do Rio de Janeiro, RJ. Brazil

Toll pathway activation during embryogenesis and during the innate immune response leads to N-terminal phosphorylation and subsequent degradation of inhibitor Cactus/IκB proteins through the proteasome. This event releases Dorsal/NFκB transcription factors that translocate to the nucleus and regulate gene expression. Cactus is also a target of proteolysis by Calpain A, producing a Cactus E10 fragment and regulating the levels of free Cactus that may interact with the Toll pathway. However, structural and biophysical requirements involved in the interaction of Calpain A and Cactus remains unknown. Here, we report the structural requirements for the action of Calpain A in the control of NFκB in Drosophila. We have modeled the Calpain A:Cactus, Cactus E10 and Dorsal:Cactus complexes, to define the previously unknown domains involved for complex establishment and maintenance. Molecular dynamic simulations suggested that the N-terminal region of Cactus undergoes wide conformational rearrangements upon interaction with Calpain A. Our results indicate that Cactus acts as a modulator in the cleavage process, by approximating the cleavage site to the catalytic site of Calpain A supporting a fit induced model. In addition, our simulations indicate that the Calpain A:Cactus complex is sustained by salt-bridges formed along the Cactus alpha helix anti-parallel ankyrin repeats and Calpain A EF hands 2, 3, 5 and CBSW domains. Subsequent mutational analysis confirms our structural predictions. Functional analysis using Cactus alleles mutated at the cleavage site and catalytically-dead Calpain A mutants demonstrates that the Cactus cleavage event by Calpain A is fundamental in modulating NFκB responses triggered by Dorsal nuclear translocation. Interestingly, our results revealed an unexpected function for Calpain A independent of catalysis, suggesting that Calpain A may also modulate Toll signal transduction by sequestering Cactus. These results indicate that competition between Calpain A:Cactus and Dorsal:Cactus complexes is an important aspect in Toll responses. New CRISPR Calpain A alleles reinforce the action of Calpain A to modify the Dorsal gradient, affecting target genes during early embryogenesis, and to modulate immune responses mediated by Toll. Taken together, these results provide a mechanistic view for the structural requirements involved in Calpain A modulating NFκB signaling.

653V Analysis of pMad and Medea Expression in BMP Pathway in Drosophila with Multiple Fluorescent Proteins *Hung-Yuan (Zeke) Chen*, Gregory Reeves Texas A&M University

Morphogen gradients are important in early Drosophila embryo development. One such gradient, the BMP/Dpp gradient, patterns the dorsal region of the embryo, which induces a series of protein interactions. After BMP-like morphogen Dpp transports to the dorsal region and binds to type I receptors Thickveins (Tkv), Tkv is activated by type II receptor Punt and then phosphorylates Mothers against Dpp (Mad). The phosphorylated Mad (pMad) and Smad4 homolog Medea translocate together to the nucleus to regulate the target gene. Compared to the Dpp gradient, pMad and Medea are procedurally closer to gene regulation. However, the transport and expression of pMad and Medea are rarely discussed. Their expression depends on the morphogen concentration which is determined by the cell location. In this work, we tag eGFP and mScarlet I to Mad and Medea and mtagBFP to Histone 2A for locating the nuclei using Crispr-Cas9 techniques and analyze the embryo images after injection into flies and crossing. Finally, we then do image analysis of 14-cycle embryos to see the expression of these proteins.

654B Abl tyrosine kinase controls the distribution and propagation of cellular forces by regulating the coherence of an actin network Aravind Chandrasekaran[1, 2], Akanni Clarke[1], Philip McQueen[1], Hsiao-Yu Fa1ng[1], Garegin Papoian[2], *Edward Giniger*[1] 1) NINDS, NIH, Bethesda, MD; 2) University of Maryland, College Park, MD

Cytoplasmic signaling pathways regulate cellular morphogenesis, but how do the nanoscale dynamics of a signaling protein create structures and direct forces at the multi-micron spatial scales of a cell? We have now compared experimental measurements of actin distribution and dynamics in single axonal growth cones in vivo in the fly wing with the results of single-molecule computational simulations of actomyosin dynamics. The results suggest a simple

framework for understanding multiscale regulation of structure and force in a living cell.

We have performed live imaging of the tip of an axon – its growth cone – in vivo in the developing fly wing. This reveals that the core of the growth cone is a mass of non-polarized actin that is undergoing constant, stochastic fluctuations. Those fluctuations are the engine of growth cone motility, as we have shown that net axon advance comes from a spatial bias in the actin fluctuations that causes the network to take more and longer steps forward than steps back. The bias comes from the cytoplasmic signaling pathways that control actin polymerization and branching. Our data show that Abl tyrosine kinase, a key downstream effector of axon guidance receptors, controls the spatial spread of the actin network: increasing Abl activity causes the growth cone actin core to expand, while decreasing Abl causes the network to contract. Computational simulations now reveal that the mechanism by which Abl controls network size is mediated through modulation of the lengths of actin filaments. Activating Abl causes a net decrease in the lengths of actin filaments, reducing their ability to link the actin network together. Consequently, the network as a whole fragments and spreads out in the growth cone, just as we observe experimentally. Conversely, reduction of Abl promotes extension of actin filaments, allowing myosin-dependent contractility to act across long length scales to condense the entire actin network, again, just as we observe experimentally.

These data provide a framework for interpreting the effects not only of Abl, but of many regulators of growth cone function. Moreover, the principles revealed here should apply to many other developmental contexts that rely on cytoplasmic signaling mechanisms to control the spatial distribution of actin-dependent structures and forces.

655C Pelado, a conserved protein that regulates actin dynamics *Claudia Molina*[1], Patricio Olguín[2], Álvaro Glavic[3], Marek Mlodzik[1] 1) Icahn School of Medicine at Mount Sinai; 2) Faculty of Medicine, University of Chile; 3) Faculty of Sciences, University of Chile

Actin dynamics are essential in every cell, primarily for cell division, migration, and morphological changes. Actin polymerization can be branched or linear, and it depends on the associated regulatory proteins. There are hundreds of proteins that regulate the actin cytoskeleton dynamics. Competition for actin monomers also occurs between proteins that induce branched or linear actin polymerization. Cell specialization requires actin cytoskeleton transformation to allow the formation of cell structures, like cuticular hairs in *Drosophila*. This is a well characterized and useful study model to analyze proteins that regulate actin dynamics. Structurally, these hairs are mainly formed by linear actin filaments. A screen was performed to identify wing development regulators. A lethal mutation was found on a gene that was named *pelado*. Pelado is a protein conserved throughout the animal kingdom but has no clear function. Epithelial mutant cells for *pelado* shows hair elongation defects. This phenotype was reversed by inducing linear actin polymerization, by reducing branched actin polymerization and by increasing the amount of actin monomers. This suggests that Pelado is involved in the competition for actin monomers. A similar phenomenon occurs in hemocytes, where Pelado is essential to induce filopodia formation in a process that also requires Diaphanous and Profilin. Pelado's function was also evaluated in A549 human cell line, in a wound healing assay. It has been described that this cell type requires branched actin polymerization to achieve efficient migration. In *pelado* knockout cells, the wound closes faster and the opposite occurs if we introduce the *pelado* fly gene in those cells. Immunoprecipitation assays suggests that Pelado is part of a multiprotein complex that includes Scar/WAVE, Diaphanos and Proflin. These data indicate that Pelado's function in regulating the actin cytoskeleton is conserved, preventing branched actin polymerization at the same time as favoring linear actin polymerization, through actin monomer competition mechanisms that modulate actin cytoskeleton dynamics. To evaluate the domains of Pelado that are important for its function, a C-terminal deletion was generated that can induce filopodia formation but does not rescue hair formation. This indicates that the C-terminal portion of Pelado has a regulatory function that is essential for certain processes, including hair formation in *Drosophila*.

656A *Spd-2* gene duplication suggests cell type-specific mechanisms of pericentriolar material assembly *Ryan O'Neill*[1], Afeez Sodeinde[2], Frances Welsh[3], Carey Fagerstrom[1], Brian Galletta[1], Nasser Rusan[1] 1) National Heart, Lung, and Blood Institute, NIH; 2) Yale, New Haven, CT; 3) University of Washington, Seattle, WA

Centrosomes are the major microtubule organizing center (MTOC) of the cell, ensuring proper spindle formation and chromosome segregation during cell division. Hundreds of proteins make up the centrosome, including many proteins that form the pericentriolar material (PCM) which nucleates microtubules. The specific requirements for MTOC activity varies across cell types, a concept we are just starting to investigate and understand. In this study, we took an evolutionary cell biological approach to gain insight into cell type-specific regulation of centrosome proteins, reasoning that gene duplication could lead to the evolution of centrosome gene duplicates with cell type-specific functions. We first used BLAST to screen 35 sequenced *Drosophila* genomes for duplications of centrosome genes, finding five that were duplicated at least once. Here, we focus on the duplication of *Spd-2* in *D. willistoni*: we refer to the parental gene as *Spd-2A* and the new copy as *Spd-2B*. In *D. melanogaster*, Spd-2 is known to function by organizing PCM in neuroblasts and spermatocytes. We found that *Spd-2B* is rapidly evolving and lacks the C-terminal 116 amino acid tail of Spd-2A. To explore expression and function we made GFP-tagged *Spd-2A* and *Spd-2B* transgenes, including their *D. willistoni* native

regulatory elements, in *D. melanogaster*. Spd-2A is ubiquitously expressed, whereas Spd-2B is only expressed in spermatogenesis. Consistent with their expression, Spd-2A, but not Spd-2B, rescues *spd-2*$^{-}$ mutant neuroblast PCM. In contrast, Spd-2B rescues *spd-2*$^{-}$ mutant spermatocyte PCM, whereas even when ectopically expressed Spd-2A fails to do so. Thus, after gene duplication Spd-2A lost the ability to organize PCM in spermatocytes, leading to complementary functions for Spd-2A and Spd-2B. We further mapped the changes responsible for this difference in function, finding that removing the C-terminal tail from Spd-2A allowed it to properly organize spermatocyte PCM, whereas adding a C-terminal tail to Spd-2B prevented it from organizing spermatocyte PCM. We infer a model where the C-terminal tail of Spd-2 mediates a priming step that precedes PCM recruitment. Together, these results show that somatic and germline cells have different requirements for PCM, and suggest that Spd-2 is differentially regulated across cell types to satisfy these distinct requirements.

657B Developing tools to study the actin mesh during *Drosophila* oogenesis *Hannah Bailey*, Margot Quinlan
University of California Los Angeles, Los Angeles, CA

The process of egg development, oogenesis, is highly conserved and crucial for producing offspring. *Drosophila melanogaster* have 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 component of oogenesis in *Drosophila* is the presence of 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). The composition, organization, stabilization, and removal of the mesh remains unknown. This is, in part, due to the requirement for actin binding proteins in early oogenesis and our inability to visualize removal of the actin mesh because egg chambers expire *ex vivo* just prior to this transition. To overcome these obstacles, I am developing methods to directly observe this meshwork and study the underlying regulatory mechanisms of the mid to late oogenesis transition. Specifically, I am developing methods for long-term *in vivo* imaging of oogenesis in *Drosophila melanogaster*. In principle, this approach will make possible study of mesh removal. Studying the roles of Spir and Capu has also been challenging due to differences in endogenous temporal control and the tools available, including the commonly used drivers of the bipartite GAL4/UAS system. I am developing improved drivers for the GAL4/UAS system to better match endogenous expression timing, allowing for careful characterization of these actin nucleators *in vivo*. Lastly, using the Auxin Inducible Degradation (AID) system, I am testing candidates to identify mesh components and regulators. Altogether, my work will lead to a greater understanding of dynamic actin rearrangements during development and facilitate detailed characterization of the actin mesh that can translate to studies in other organisms.

658C Dynein acts to cluster glutamate receptors and traffic the PIP5 kinase, Skittles, to regulate postsynaptic membrane organization at the neuromuscular junction *Amanda L. Neisch*[1], Thomas Pengo[1], Adam W. Avery[1,2], Min-Gang Li[1], Thomas S. Hays[1] 1) University of Minnesota, Minneapolis, MN; 2) Oakland University, Rochester, MI

Cytoplasmic dynein is essential in motoneurons for retrograde cargo transport that sustains neuronal connectivity. Little, however, is known about dynein's function on the postsynaptic side of the circuit. Using genetic, immunolocalization, and electrophysiology studies, we have identified distinct postsynaptic roles for dynein at neuromuscular junctions (NMJs). We have found that dynein punctae accumulate specifically on the postsynaptic side of glutamatergic synaptic terminals. Postsynaptic dynein is required for the localization of $PI(4,5)P_2$, a phospholipid membrane component, and a number of membrane-associated proteins including components of the spectrin cytoskeleton. Skittles, a phosphatidylinositol 4-phosphate 5-kinase that produces $PI(4,5)P_2$ to organize the spectrin cytoskeleton, also localizes specifically to glutamatergic synaptic terminals and this localization is dynein dependent. Further, depletion of postsynaptic dynein results in enlarged ionotropic glutamate receptor (iGluR) clusters and an increased amplitude and frequency of mEJPs. $PI(4,5)P_2$ levels do not affect iGluR clustering and dynein does not affect the levels of iGluR subunits at the NMJ, suggesting a unique transport independent function of dynein at the NMJ in clustering iGluRs. As dynein punctae closely associate with iGluR clusters, we propose that dynein physically stabilizes iGluRs at the postsynaptic membrane for proper synaptic transmission.

659A β_H-spectrin Recruits PP2A^{Waldorf} to Crumbs where it Regulates Growth and Apical Domain Stability In *Drosophila* Kristen Browder[1,2], Seung-kyu Lee[1,3], Elizabeth Klipfell[1], Mark Travor[1], Katelyn Wolfgang[1], *Claire Thomas*[1] 1) Penn State, University Park, PA; 2) Genentech, South San Francisco, CA; 3) National Institute of Aging (NIH/NIA/IRP), Baltimore, MD

Spectrin is a large F-actin crosslinking protein that most famously forms 2D networks in association with the plasma membrane of red blood cells. This 'membrane skeleton' confers cell shape and membrane strength during the rigors of circulation. In NON-erythroid tissues, spectrin has additional roles in the endomembrane system. We have previously shown that the apically polarized $\beta_H$spectrin (β_H), encoded by the *karst* locus in *Drosophila*, is required for the stability of several apical proteins, through the promotion of endosomal recycling to the plasma membrane - so called, 'dynamic protein stabilization'. The apical protein determinant Crumbs recruits β_H to the apical membrane and is itself trafficked in a β_H-dependent manner. β_H binds to the Hippo/Warts pathway (HWP) regulator Expanded, which mediates Crumbs

crosstalk to the HWP.
Here we report that a yeast 2-hybrid (Y2H) screen identified the PP2A substrate-specificity subunit Waldorf (a PP2A-PR72/B'' isoform) to be a binding partner of $\beta_H$spectrin. Waldorf binds to β_H *via* a short, conserved sequence in its C-terminal globular domain. Genetic interaction and molecular epistasis experiments strongly suggest that PP2A with the Waldorf specificity-subunit bound to it ($PP2A^{Waldorf}$) acts as a negative regulator of Crumbs by acting to displace aPKC from Crumbs. Consistent with this notion, mutant versions of Crumbs lacking target residues for aPKC in the FERM-domain binding site do not respond to changes in Waldorf levels. Knockdown of Waldorf leads to reduced growth suggesting that Crumbs crosstalk with the HWP is a primary target of this homeostatic regulation. Overexpression of Waldorf specifically destroys the apical domain suggesting that Waldorf may also play a role in modulating the apical domain.
We also show that Waldorf modulates protein trafficking in a similar way to β_H and its previously reported partner Annexin B9 in that knockdown of Waldorf leads to an increase in Rab7-positive and acidic compartments, suggesting that $PP2A^{Waldorf}$ also normally acts by suppressing lysosomal trafficking, most likely in favor of recycling pathways.
Our results support a model in which Crumbs recruits β_H in a complex with the HWP activator Expanded, and $PP2A^{Waldorf}$ bound to β_H acts in a homeostatic fashion to limit Crumbs activation of the HWP by displacing aPKC. This in turn limits the amount of growth suppression caused by Crumbs-dependent HWP activation.

660B Cullin 3 promotes polarization of aPKC phosphorylated differentiation determinants during asymmetric neuroblast division *Cheng-yu Lee*, Hideyuki Komori, Noemi Rives-Quinto, John Bugay Univ Michigan

The aPKC/Par-6 complex is widely used in polarizing cortical localization of protein determinants that promote proper specification of cell identity and cell functionality. The prevailing model suggests that aPKC phosphorylation displaces its target proteins from the aPKC/Par-6 cortical domain into cytoplasm by perturbing their interactions with phospholipids or adaptors, leaving unphosphorylated proteins enriched in the opposite cell cortex. It remains unclear if phosphorylation-induced exclusion from the cortex is a generalizable mechanism for aPKC-mediated polarization of numerous downstream proteins during development and homeostasis. During asymmetric neuroblast division, aPKC kinase activity in the apical cortex regulates basal localization of Notch antagonists including Numb and their segregation into neuroblast progeny where they promote differentiation by downregulating Notch signaling. In contradiction of the prevailing model, we found that aPKC kinase activity levels positively correlate with polarized Numb accumulation in the basal cortex of mitotic neuroblasts. Analyses of novel fly or human *numb* alleles that encode missense mutations at two conserved aPKC phosphosides indicate that the phosphomimetic but not the non-phosphorylatable form of Numb protein asymmetrically localizes to the basal cortex of mitotic neuroblasts. These results indicate that aPKC phosphorylation positively induces polarization of Numb. We screened for genes that are required for Numb segregation during asymmetric neuroblast division in *numb*-hypomorphic brains, which show a mild supernumerary neuroblast phenotype due to reduced Numb activity in neuroblast progeny destined to differentiate. We identified the *cullin 3* (*cul3*) gene as a novel regulator of Numb polarization in mitotic neuroblasts. Mitotic *cul3*-null neuroblasts show aPKC and Numb localized uniformly throughout the cortex, reducing Numb levels in neuroblast progeny and leading to their reversion into supernumerary neuroblasts due to defects in downregulation of Notch signaling. Consistent with defects in aPKC-induced Numb polarization, increased aPKC kinase activity levels restore Numb polarization in the basal cortex of mitotic *cul3*-null neuroblasts and restore differentiation in their progeny. We propose that the non-proteolytic function of Cul3 promotes aPKC phosphorylation-induced Numb polarization in mitotic neuroblasts by facilitating efficient Numb phosphorylation by aPKC.

661C Unraveling Positive and Negative Feedback in Planar Cell Polarity *Alexis Weiner*, Kaye Suyama, Jeffrey Axelrod Stanford University

Planar Cell Polarity (PCP) polarizes cells along an axis parallel to the tissue plane, and this results in organized cell polarity across entire tissues. PCP includes both positive and negative feedback mechanisms to cause the polarization of two core protein complexes. However, the interdependency between molecular players and lack of genetic techniques has left the role of feedback mechanism mired in mystery. To uncover a more detailed mechanism of polarization we have developed a novel genetic 'Velcro' approach to force molecular players in the pathway to cell-cell junctions. This method allows us to parse the specific steps of polarization and differentiate between the role of positive and negative feedback in PCP. It also lets us ask the important evolutionary question of whether cells within PCP retain the single cell system of polarization when we use this tool together with a mutant that removes cell-cell PCP communication. Using the novel 'Velcro' tool, we have shown by proof of concept that forcing a single component, Dsh, to cell-cell junctions at the clonal boundary we can reverse cell polarity.

We can dissect the role of clustering as the mechanistic function underlying positive feedback by expressing clustering mutants of Dsh, Pk and Fmi. We have engineered flies expressing proteins that are oligomerization deficient and will position them using 'Velcro'. This will determine if self-assemblies are required for polarization. Finally, we can test if this is also required for cell autonomous polarization. These experiments will reveal the role of positive feedback during cellular polarization and if PCP retains the ability of single cells to use this positive feedback to polarize autonomously.

We will also tackle the proposed long-range negative feedback and role of 'mutual' exclusion in the segregation of proximal and distal complexes in PCP. Using our novel 'Velcro' technique, we are testing if proximal proteins such as Vang and Pk are excluded when Dsh is forced to localize asymmetrically at clonal boundaries. We also will ask if this exclusion requires clustering by introducing oligomerization deficient Dsh mutants. Second, we will ask if this exclusion can happen cell autonomously by removing cell-cell connections with the Fmi mutant. Furthermore, we will investigate the minimal requirements for this exclusion by removing other molecular players such as Fz, Dgo, Vang, and Pk. Lastly, we will attempt to perform the same exclusion experiments by forcing Pk, the proximal counterpart to Dsh. We will evaluate if distal complexes are excluded in alignment with the idea of 'reverse' mutual exclusion. Ultimately this will complement the first set of experiments and reveal the role of long-range negative feedback in PCP signaling. With these tools and experiments, we aim to address the previously inaccessible questions concerning the contributions of positive and negative feedback in PCP.

662A The Establishment and Maintenance of Centrosome Asymmetry in Neural Stem Cells *Roberto Segura*, Clemens Cabernard University of Washington

Asymmetric cell division (ACD) is an evolutionarily conserved process where proteins, organelles, and mRNAs are unequally partitioned between daughter cells. This maintains the stem cell population, where progenitor cells undergo ACD to produce a self-renewing stem cell and a differentiating daughter cell. Organelles, such as centrosomes, can be partitioned asymmetrically between daughter cells, and this is observed in many diverse cell types. In *Drosophila* neural stem cells, called neuroblasts, the differentiating ganglion mother cell receives the older mother centrosome, while the renewing neuroblast receives the younger daughter centrosome. This biased inheritance is determined by differences in molecular identity, which is mediated by the phosphorylation activity of Polo kinase. Preliminary data has implicated the involvement of protein phosphatase 4 (PP4) in this cascade, and the extent of PP4's molecular involvement in this process is not fully understood. These molecular identities are speculated to impact the other factors during ACD. Additionally, centrosomes retain distinct clusters of mRNA, and this retention is theorized to be facilitated by the molecular identity of the centrosome. However, the spatiotemporal mechanisms that regulate centrosome asymmetry and its functional impact are unknown. I hypothesize that (1) dephosphorylation of cytoskeletal proteins, such as gamma-tubulin, contribute to the establishment and maintenance of centrosome asymmetry, and (2) biased mRNA localization is dependent on centrosome asymmetry. To address these hypotheses, I will utilize fluorescent *in vivo* imaging and novel optogenetic methods to elucidate the spatiotemporal regulation and functional importance of centrosome asymmetry. These experiments will reveal the regulation of this process within the context of ACD, which will broaden our understanding of its importance in other processes, such as stem cell differentiation and the onset of cancer.

663B Regulated demolition in muscle remodeling: a T-tubule membrane disassembly pathway maintains muscle function *shravan girada*, jen Nguyen, Tzu-Han Lin, Amy Kiger Section of Cell and Developmental Biology, Division of Biological Sciences, University of California, San Diego

Muscles are complex cells with specialized structures for contraction. While arrays of sarcomeres perform contraction, it is the network of plasma membrane invaginations, called Transversal (T)-tubules, that coordinate sarcomere contractions. We study T-tubule requirements in body wall muscles of Drosophila at different growth stages, which allows live imaging of the membrane network. We uncovered an endogenous requirement for *shibire,* a Dynamin large GTPase, in the initiation of T-tubule disassembly within a wildtype developmental muscle remodeling program. Consistent with its role in disassembly, overexpression of *shibire* led to inappropriate T-tubule fragmentation and defective muscle function for larval mobility. These results indicate the importance for regulation of a normal dynamin function in T-tubule disassembly that may also hint at how dominant mutations in a conserved human DNM2 lead to centronuclear myopathy (CNM).
Previously, we implicated Class II PI3-kinase, Pi3K68D (or PI3KC2), in abdominal muscle remodeling defects associated with loss of Mtm PI3-phosphatase, a homolog of human MTM1 linked to a recessive (X-linked) form of CNM. We now identify a requirement for *Pi3K68D in* T-tubule disassembly at initiation of regulated muscle remodeling. Remarkably, disruption of *Pi3K68D* function also prevented the ectopic T-tubule fragmentation induced by *shibire* overexpression. Interestingly, mammalian PI3KC2A activity has been shown to recruit DNM2 for its role in endocytic vesicle formation, raising the question whether a shared pathway is also involved in T-tubule fragmentation. Our current studies unveil *Pi3K68D* phosphoinositide dependence, sites of Pi3K68D and Shibire function and the contributions of other endocytic pathway members in T-tubule disassembly. Altogether, our results suggest shared elements between the mechanism for dynamin-mediated endocytosis and T-tubule membrane disassembly and elevate the prospects of human PI3KC2A-targeted therapies for both dominant DNM2- and recessive MTM1-related myopathies.

664C Systematic functional analysis of Rab GTPases in neuronal development and maintenance *Ilsa-Maria Daumann*, Friederike Kohrs, P. Robin Hiesinger Freie Universitaet Berlin

Neurons are morphologically elaborate and long-living cells. Both features put special demands on membrane trafficking. Rab GTPases are regulators of membrane trafficking and the *Drosophila* genome encodes 26 *rab* genes. All *rabs* are expressed in the nervous system and 13 of the 26 *rabs* exhibit nervous system-enriched expression patterns. To facilitate systematic functional analyses of neuron-specific membrane trafficking, we have generated a complete *rab* null mutant collection. Remarkably, the complete loss of any of the 13 nervous system-enriched Rabs did not cause obvious defects concerning fly survival, fertility, or morphology under laboratory conditions. However, under challenging conditions including continuous neuronal stimulation or temperature variation all *rab* mutants revealed specific sensitivities affecting development, function, or maintenance of the nervous system. Our observations suggest that the majority of Rabs serve modulatory functions that ensure robustness of development or function to challenging environmental conditions.
We followed up on our initial systematic characterization with a more detailed analysis of four neuronal or neuron-enriched *rabs*: *rab26*, *rab19*, *rabX1* and *rabX4*. Rab26 has previously been proposed to link synaptic vesicle recycling and autophagy. However, our characterization of the *rab26* mutant revealed no autophagosomal or synaptic vesicle defects in flies. Instead, we found an activity-dependent role of Rab26 in receptor trafficking at cholinergic synapses. Our analyses of *rab26, rab19, rabX1* and *rabX4* revealed synthetic lethality for double mutants of *rabX1* with each of the other three, but no other double mutant combination. We will present an update on our characterization of the functional contributions of each of these *rabs*.

665A The STRIPAK complex and microtubule protein transport in *Drosophila* muscle tissue *Yungui Guo*, Erika Geisbrecht 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 macroautophagy. 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-Hsc70-4 complex plays a role in the autophagic clearance of proteins. However, the mechanism of how damaged proteins get transported to the sites of degradation is not clear. Based upon literature in neuronal cells that describe a role for the Striatin Interacting Phosphatase and Kinase (STRIPAK) complex in the axonal transport of autophagosomes, we propose the hypothesis that the STRIPAK complex may be required to transport proteins along microtubules (MTs) to the lysosome in *Drosophila* larval muscles. To test for a possible role of the supramolecular STRIPAK complex in BAG3-mediated transport, we have employed a sensitized genetic assay using RNA interference (RNAi) technology. Thus far, our data shows that *stv* genetically interacts with genes that encode for some members of the STRIPAK protein complex, including Striatin interacting protein (Strip1), MOB kinase activator 4 (Mob4), and Connector of kinase to AP-1 (Cka). Moreover, preliminary observations suggest perturbations in the MT network within muscle cells upon RNAi knockdown of STRIPAK complex members. This project will give us a better understanding of how the NUAK-Stv-Hsc70-4 complex functions with STRIPAK complex in the autophagic clearance of protein and maybe lead to finding some potential drug targets for treating protein aggregate diseases in human.

666B MDIS, a mitochondrial DNA exonuclease enforces uniparental inheritance of mitochondrial genome *Zhe Chen*[1], Christian A Combs[1], Yong Chen[1], Annie Lee[2], Hong Xu[1] 1) National Institutes of Health; 2) Walter Reed National Military Medical Center

Mitochondrial genome is exclusively transmitted through maternal linage in animals. The uniparental inheritance was once regarded as a passive outcome of distinct cytoplasmic contents of eggs and sperms. Recent studies demonstrated active mechanisms to remove mitochondrial DNA (mtDNA) during spermatogenesis in various species. However, the physiological significance of mtDNA clearance, or mitochondria uniparental inheritance in general remains a mystery and the factors involved in this process are largely unknown. Using proximity-labeling based proteomic screen, we recovered a putative mitochondrial nucleoid protein MDIS (mitochondrial DNA in sperm), encoded by *CG12162* locus. MDIS is highly enriched in *Drosophila* testis, and specifically in late spermatogenesis stages, when the mtDNA clearance takes place. *MDIS* null flies are male semi-sterile, otherwise completely healthy. Spermatogenesis progresses normally in *MDIS* flies *but* produces immotile mature sperms that often contain multiple copies of mtDNA nucleoids, indicating that MDIS is required for mtDNA clearance. *In vitro* assay suggests that MDIS is an exonuclease that degrades both single-stranded DNA and double-stranded DNA with overhangs. Importantly, ectopic expression of a mitochondrially targeted *E.coli* exonuclease III in *MDIS* flies, effectively removes mtDNA remnants and largely restores male fertility, demonstrating that failure to eliminate mtDNA causes immotile sperms and male infertility. Surprisingly, we observed pronounced nuclear DNA fragmentations in mature sperms of *MDIS* flies, suggesting a potential detrimental consequence of persisting mtDNA in mature sperms. Our results demonstrate that MDIS play essential roles in preventing the transmission of paternal mtDNA to the offspring, and in safeguarding the nuclear genome integrity. MDIS, to our knowledge, represents the first-ever identified factor that specially enforces maternal inheritance of mitochondrial genomes. This will allow future studies to understand physiological significances and underlying mechanisms of this highly conserved, yet mysterious phenomenon.

667C Roles for *CG5755,* a *SLC25A46* ortholog, in mitochondrial morphogenesis during *Drosophila* spermatogenesis *Claire Olson*, Caroline Phan, Tommy Mason, Kyle Kowalewski, Sam Kavarana, Vivienne Fang, Karen G Hales Davidson College

Drosophila spermatogenesis involves significant morphological changes in mitochondria and thus provides a useful system to study genes that regulate mitochondrial movement and shaping. The *Z2-3738* recessive male sterile fly strain, originating from the Zuker collection, displays defects in spermatogenesis, including male sterility and disorganized sperm bundles with scattered short mitochondrial aggregates, due to a nonsense mutation in *CG5755*, a testis-specific gene. Its paralog, *CG8931*, is expressed in most other tissues. The human ortholog, *SLC25A46*, a member of a mitochondrial solute carrier family, has been shown to localize to the mitochondrial outer membrane and function in mitochondrial fission. *SLC25A46* has been implicated in several human neurodegenerative diseases; thus, elucidating the function of its *Drosophila* orthologs may provide valuable insight into these conditions. Here, we characterize the function of *CG5755* by exploring its localization, interactions, and putative role in the nervous system as proposed by others. We observed localization of a CG5755-GFP transgenic protein in the mitochondria of sperm bundles and potentially in the nebenkern. We investigated genetic interactions of *CG5755* with genes involved in mitochondrial morphology, including the fusion gene *fzo* and cristae shaping gene *mic60*, and observed a synthetic phenotype in combined *Z2-3738* and *fzo* mutants, suggesting that CG5755 may function in a conserved mitochondrial fusion pathway with *fzo*. Additionally, although current RNA-seq data report *CG5755* as a testis-specific gene, another group reported evidence of neurological defects in neuron-specific *CG5755* knockdown flies. We conducted experiments to determine whether CG5755-GFP localizes to nervous system tissues and whether *Z2-3738* flies show defects in locomotor function. Together, these results confirm the role of *CG5755* in mitochondrial shaping in spermatogenesis, provide preliminary evidence about its function in mitochondrial dynamics and its interactions with other relevant genes, and demonstrate whether it is functionally active in the nervous system.

668A Moonlighting of the Golgi protein, Gorab, at the centriole is regulated by its high affinity for centriolar protein Sas6 *Levente Kovacs*[1], Jennifer Chao-Chu[2], Sandra Schneider[2], Agnieszka Fatalska[2,3], Magdalena M Richter[2], Emma Stepinac[4], Marco Gottardo[5], George Tzolovsky[2], Nikola S Dzhindzhev[2], Maria Giovanna Riparbelli[5], Giuliano Callaini[5], Michal Dadlez[3], Gang Dong[4], David M Glover[1,2] 1) Division of Biology and Biological Engineering, California Institute of Technology, Pasadena, California, USA; 2) Department of Genetics, University of Cambridge, UK; 3) Laboratory of Mass Spectrometry, Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland; 4) Max Perutz Labs, Vienna BioCenter, Vienna, Austria; 5) Department of Life Sciences, University of Siena, Siena, Italy

Centrioles are evolutionarily conserved organelles required to assure the fidelity of cell division, motility and signaling. Although the majority of key proteins ensuring proper centriolar structure, duplication and maturation have been described, several new candidates have emerged from protein interaction studies. In a search for interacting partners of the core centriole cartwheel protein, Sas6, of Drosophila, we recently identified Gorab, better known as a Golgi protein mutated in the human disease Gerodermia osteodisplastica. We discovered that Gorab is a moonlighting protein – whereas dimeric Gorab localizes to the trans-Golgi, monomeric Gorab directly interacts with Sas6 dimers at the centriole's core where it is essential for centriole duplication and symmetry. Here we show that Gorab's high binding affinity to the limited amount of centriolar Sas6 is a key determinant of Gorab's localization. Thus, upon Gorab overexpression, there is no increase in its levels at centrioles and the surplus Gorab associates with the trans-Golgi. Meiotic spermatocytes are particularly sensitive and moderate Gorab overexpression results in a Golgi-related cytokinesis defect and consequently male sterility. Upon Sas6 overexpression, however, the amount of Gorab dramatically increases both in the centrioles and in non-centriolar Sas6 aggregates and Golgi-associated Gorab decreases. Moreover, targeting elevated levels of Sas6 to mitochondria re-localizes endogenous Gorab to this organelle while depleting Gorab at the Golgi. In line with these observations, *in vitro* interaction assays reveal that Sas6 can disrupt preformed Gorab homodimers and disassociate Gorab from its Golgi-associated interactor Rab6. Strikingly, Sas6 overexpression can rescue the cytokinesis defects of Gorab-overexpressing males and restore their fertility. Thus, the high affinity of the Sas6 dimer for Gorab monomers ensures that Gorab gives priority to its moonlighting function at the centriole allowing remaining Gorab to dimerize and localize to the Golgi for its official duties.

669B Essential functions of *gish* in nuclear positioning during early embryogenesis *Lingkun Gu*, Mo Weng University of Nevada, Las Vegas

Nuclei are often actively positioned for specialized cellular functions or developmental purposes and failures in correct positioning can lead to dysfunctions and defects. Early embryos of Drosophila melanogaster are tasked with positioning hundreds of nuclei because the embryogenesis begins with 13 synchronized nuclear divisions within a syncytium. Although nuclei initially localize in the anterior half of the embryo, during nuclear cycle 4-6, they are evenly spread along the anterior-posterior axis in an actomyosin-dependent process called axial expansion. Then, during cycle 7-9, nuclei migrate and distribute evenly in the syncytial cortex, followed by the last four nuclear divisions. The even nuclear spacing ensures that cells of similar sizes are generated during the cleavage stage. However, the molecular mechanisms of axial expansion and cortical migration are still poorly understood. Here, we identify a plasma membrane-associated

kinase *gilgamesh (gish)* as an important player in this complex process. *gish*-depleted embryos show defects in axial expansion: nuclear spreading to the posterior half of the embryo is delayed and the nuclei preferentially localize to the anterior half. Interestingly, the nuclei also show premature localization to the syncytial cortex, suggesting *gish* may be involved in the positioning of active myosin or in a mechanism that balances myosin-induced cytoplasmic flow. During the last four nuclear divisions on the cortex, *gish* depletion leads to defects in mitotic furrow formation, which further disrupts nuclear spacing and is accompanied by desynchronization of the nuclear cycle. In summary, we show that *gish* is required for the even positioning of nuclei in fly syncytium at multiple stages.

670C Why are axonal endoplasmic reticulum tubules so narrow? *Kishen Chahwala*, Cahir O'Kane University of Cambridge

Axonal endoplasmic reticulum tubules are much narrower in axons than in most other locations. But what does this mean for lumenal protein diffusion? To test whether the small lumen diameter is limiting for protein diffusion, we compared the rate of diffusion of a GFP-tagged lumenal protein through wildtype larval *Drosophila* axonal ER tubules, with diffusion through mutant axonal ER tubules with a larger diameter, in a triple mutant lacking the ER-shaping proteins, Rtnl1, ReepA and ReepB. We performed FRAP experiments on axons to look at fluorescence recovery and thus the freedom of GFP to diffuse through the ER lumen.

We found that lumenal diffusion of GFP was significantly faster in the larger mutant ER tubules. The time to half recovery in wider axonal ER tubules (lacking Rtnl1, ReepA and ReepB) was approximately half as long as in wildtype.

The result implies that narrow axonal ER tubule diameter limits lumenal protein diffusion. This leaves open the question of why it might be important to limit lumenal ER diffusion along axons, for example, whether the diffusion of the much smaller (relative to GFP) Ca^{2+} ion is also constrained, and whether altered ER lumenal diffusion might be a factor in the pathology of diseases like Hereditary Spastic Paraplegia that are caused by mutations in ER-shaping proteins.

671A EMC is required for biogenesis and membrane insertion of Xport-A, an essential chaperone of Rhodopsin-1 and the TRP channel Catarina Gaspar[1,2], Ligia Vieira[1], Cristiana Santos[1], John Christianson[3], David Jakubec[4], Kvido Strisovsky[4], Colin Adrain[2,5], *Pedro Domingos*[1] 1) Instituto de Tecnologia Química e Biológica da Universidade Nova de Lisboa (ITQB-NOVA); 2) Membrane Traffic Lab, Instituto Gulbenkian de Ciência (IGC); 3) University of Oxford; 4) Czech Academy of Sciences; 5) Queen's University

The ER membrane complex (EMC) is required for the biogenesis of a subset of tail anchored (TA) and polytopic membrane proteins, including Rhodopsin-1 (Rh1) and the TRP channel. To understand the physiological implications of EMC-dependent membrane protein biogenesis, we performed a bioinformatic identification of *Drosophila* TA proteins. From 254 predicted TA proteins, screening in larval eye discs identified 2 proteins that require EMC for their biogenesis: fan and Xport-A. Fan is required for male fertility in *Drosophila* and we show that EMC is also required for this process. Xport-A is essential for the biogenesis of both Rh1 and TRP, raising the possibility that disruption of Rh1 and TRP biogenesis in EMC mutants is secondary to the Xport-A defect. We show that EMC is required for Xport-A TMD membrane insertion and that EMC-independent Xport-A mutants rescue Rh1 and TRP biogenesis in EMC mutants. Finally, ER resident, N196-glycosylated Rh1 TMD1-5 accumulates in Xport-A and EMC6 mutants, revealing a role for Xport-A in a glycosylation-dependent triage mechanism during Rh1 biogenesis in the endoplasmic reticulum.

672B Developing a *Drosophila* genetic screen for mutations that disrupt axonal ER organization *Nishani Jeyapalan*, Cahir O'Kane Genetics Department, University of Cambridge

Developing a *Drosophila* genetic screen for mutations that disrupt axonal ER organization
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Axonal endoplasmic reticulum (ER) consists of a continuous tubular membrane network. Its continuity and potential as a channel for long-distance communication have earned it the description "a neuron within a neuron". Mutations affecting ER-modeling proteins can cause the axon degeneration disease, hereditary spastic paraplegia (HSP), implying the importance of this network in axon maintenance. While the assembly, distribution, transport, and density of the network must be regulated to meet the needs of the neuron, we understand hardly anything of this regulation. To address this, our goal is to identify novel proteins involved in organizing and maintaining the ER network, using targeted CRISPR/CAS9 mutagenesis.

To allow easy visualization of axonal ER for screening, we identified *Drosophila* lines expressing GAL4 in small numbers of motor neurons innervating the adult leg, and used them to express fluorescent ER and plasma membrane markers, observed through undissected cuticle in adult legs. To allow tissue-specific CRISPR/CAS9 mutagenesis in the labelled motor neurons, we introduced a *UAS-CAS9* construct into these flies, targeting CAS9 expression only to the labelled

motor neurons utilising the *Drosophila* GAL4 system. Crossing this stock to any transgenic guide-RNA (gRNA)-expressing line allows tissue-specific somatic mutagenesis in the labelled neurons, and screening for axonal ER phenotypes as a consequence of tissue-specific gene disruption.

We have performed a pilot mutagenesis screen to observe axonal ER phenotypes in CAS9-mutated *Drosophila* motor neurons. We selected a small library of gRNAs, targeting a small selection of genes encoding ER-associated proteins, HSP protein orthologs, and motor proteins. We crossed these gRNA lines to the line expressing ER and PM markers and CAS9 in leg motor neurons, and tested the progeny for alterations in ER distribution in adult leg femoral motor axons.

Confocal screening showed a highly discontinuous axonal ER network as well as differences in ER and PM average intensities using several of these gRNA lines, in contrast to the more uniform continuous ER structure observed in control motor axons.

Having shown proof-of-principle for detecting axonal ER phenotypes caused by gRNAs in motor axon ER, we are now individually investigating gRNAs that cause axonal ER phenotypes via *in vivo* larval imaging, to ascertain whether axonal ER dynamics are also affected. Furthermore, we can now expand the pilot screen to encompass genes involved in several biological pathways, testing the roles of various gRNAs on axonal ER architecture.

673C A Dominant modifier Screen for Genetic Interactor of Jagunal in the Drosophila compound eye *Gerson Ascencio*, Judy Abuel , Jorge Inojoza San Francisco State University, San Francisco, CA

The endoplasmic reticulum (ER) is a continuous network of membrane tubules and flattened cisternae involved in protein and lipid synthesis, protein secretion, and post-translational protein modification. A recent study showed that the ER is partitioned asymmetrically in proneuronal cells during mitosis in early Drosophila embryos prior to cell fate determination. Furthermore, this asymmetric ER partitioning relies on the highly conserved ER transmembrane protein Jagunal (Jagn), however, the molecular pathway that drives Jagn-dependent ER partitioning is currently unknown. Here, we hypothesize that Jagn interacts with pathways involving cell fate selection to drive the generation of neuronal cell diversity. To identify possible genes that interact with Jagn, we performed a dominant modifier screen in the Drosophila compound eye. Expression of JagnRNAi in the Drosophila compound eyes results in a rough eye phenotype in 80% of the eyes examined. Based on this, we crossed a collection of deficiency lines (DF) covering the entire 3rd chromosome and examined for either enhancement or suppression of the rough eye phenotype. We have identified ten suppressors and twelve enhancers of Jagn-induced rough eye lines phenotype. We examined these DF lines and selected eight genes involved in functions such as organelle assembly, microtubule attachment, and organelle movements. We then performed a secondary dominant modifier screen in the Drosophila compound eye, using mutants of the selected target genes and mutant gene Dally gave us a similar rough eye phenotype percentage. Dally is important for neuron projection morphogenesis, and positive regulation of signal transduction. We hypothesize that Dally can be working with Jagn as a cell fate determinant. Targets will provide important insight into the molecular pathway involved in ER organization and partitioning.

674A Endosomal maturation in *Drosophila* nephrocytes depends on a trimeric Rab7 GEF complex *Maren Janz*[1], Lena Dehnen[1], Jitender Kumar Verma[2], Olympia Ekaterini Psathaki [3], Lars Langemeyer[2], Florian Fröhlich[4], Jürgen J. Heinisch[5], Heiko Meyer[1], Christian Ungermann[2], Achim Paululat[1] 1) University of Osnabrück, Department of Biology and Chemistry, Zoology and Developmental Biology; 2) University of Osnabrück, Department of Biology and Chemistry, Biochemistry; 3) University of Osnabrück, Center of Cellular Nanoanalytics, Integrated Bioimaging Facility Osnabrück (iBiOs); 4) University of Osnabrück, Department of Biology and Chemistry, Molecular Membrane Biology; 5) University of Osnabrück, Department of Biology and Chemistry, Genetics

Drosophila nephrocytes are specialized cells displaying highest endocytic activity. As a functional adaptation they display enormous cell membrane expansions and membrane tubulation where the endocytic active site is situated. The huge size of the cells also ensures enormous storage capacity, e.g. for degraded proteins or metabolic components[1]. We use nephrocytes to investigate the trimeric Rab7 guanine nucleotide exchange factor (GEF) complex Mon1-Ccz1-Bulli. During endocytosis, Rab5 decorated early endosomes mature into Rab7 positive late endosomes. Subsequently, fusion with lysosomes leads to acidification and degradation of the lysosomal content/cargo. To function on membranes, Rab proteins are activated by GEFs and further inactivated by GTPase activating proteins (GAPs). Maturation of early endosomes to late endosomes and the fusion with lysosomes also depend on the two heterohexameric tethering complexes CORVET and HOPS. The CORVET complex is an effector of Rab5 whereas HOPS interacts with activated Rab7[2]. In *Drosophila*, a trimeric Rab7 GEF complex, containing Mon1, Ccz1 and Bulli is active. We have shown that the absence of Bulli results in impaired endosomal maturation and enlarged Rab7 positive and negative endosomes with clustered Rab5 inside[3]. Furthermore, the lack of either Mon1 or Ccz1 reveals defective Rab5 and Rab7 localization and internalization of Rab5 into endosomes. Bulli has been identified in insects[3] and mammalian cells[4], indicating that the trimeric GEF is common in metazoan.

Overall, our data demonstrate the importance of the trimeric Rab7 GEF complex for proper localization of Rab5 and Rab7 in *Drosophila*. Our work aims to provide fundamental insight into the regulation of the endocytic pathway via this complex in nephrocytes. Especially the mechanism leading to Rab5 clustering in mutant flies will be investigated in future studies.

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675B Peroxisome metabolism in enterocytes regulates the diet-gut-brain axis and lead to neurodegeneration *Francesca Di Cara*, Stephanie Makdissi, Julia Kalinowski, Eden Bishop, Alex Liaukovich, Smitha George Dalhousie University

Peroxisomes are metabolic organelles that contribute to maintain the healthy metabolic status of the cell.Emerging evidence suggests that peroxisomes contribute in different ways to the function, development and survival of different tissues. Recent studies demonstrated that the peroxisome is essential in intestinal epithelial cells for the metabolic status of the organism, to regulate tissue inflammation and to maintain host-commensal/pathogen interactions in the gut. Considering the importance of gut-derived signaling for interorgans communication such as the gut-brain axis, we performed transcriptomic and metabolomic analyses to determine whether peroxisome metabolism in the gut controls secretion of trophic factors that contribute to neuronal health in adult flies. Integration of metabolomic and peptidomic analyses of the hemolymph integrated to transcriptomic and metabolomic analyses of control guts and peroxisome-depleted guts, revealed that peroxisomes modulate the production and secretion of gut-specific neuropeptides and inflammatory lipids in the circulation and in turn affect the gut-brain communications. Alteration of peroxisomal β-oxidation causes lipotoxicity in enterocytes triggers systemic release of inflammatory lipids (Cer22/Cer18) and inhibits the secretion of trophic neuropeptides such as Bursicon and Npl1 that are necessary to promote metabolic adaptation and stress responses in the organism. The transcriptional inhibition of these genes and the accumulation of inflammatory lipids together lead to brain inflammation, locomotor defects and reduce animal lifespan. Treatment with the free fatty acids scavenger, Niacin, rescues the described phenotypes in adult flies that have depleted peroxisomes in the enterocytes. Conversely, treatment with the established inhibitor of peroxisomal β-oxidation Thioridazine, or feeding an high fat diet lead to brain inflammation, dopaminergic neurons death, locomotion defects and reduces lifespan in control flies.
Considering that emerging evidence suggests that changes in diet-gut-brain axis influence the metabolic and inflammatory status of the neurons that is linked to Neurodegenerative Diseases (NDs) and that mild mutations in peroxisome genes are associated to the onset of NDs, we believe this work advanced our understanding of the the impact of peroxisomal metabolism on gut-brain axis, neuro-inflammation and the onset of NDs.

676C A neuroprotective role of select peroxisome proteins at the fat body of Drosophila melanogaster *Kazuki Ueda*[1], Matthew Anderson-Baron[1,2], Nathan Hoeven[3], Julie Haskins[1], Andrew Simmonds[1] 1) Department of Cell Biology, Faculty of Medicine and Dentistry, University of Alberta, Edmonton, AB, Canada T6G 2H7; 2) Future Fields Ltd. Edmonton, AB, Canada T5H 0L5; 3) Ross Shepard High School, Edmonton, AB, Canada T5M 2P2

A systemic lipid homeostasis is crucial for proper functioning and development of organ systems. Peroxisome is a membrane-bound organelle and is one of the central hubs of lipid metabolism. A congenital, autosomal recessive disorders affecting one of 14 core peroxisome genes, *Peroxins* (*Pex*), leads to a spectrum of peroxisome biogenesis disorders (PBDs) exhibiting neurological deficits and developmental delays in children currently with palliative treatments as only available options.
Peroxisomes are thought to associate and coordinate with lipid droplets (LDs) (cellular fat stores) to regulate β-oxidation of fatty acids and promote synthesis of plasmalogens and docohexanoic acids. Our lab has recently identified peroxisome-independent functions of two Pex proteins (or Peroxins), Pex13 and Pex14, in promoting lipid storage at LDs in *Drosophila melanogaster*. In particular, we observed that Pex14 prevented localization of hormone sensitive lipase (Hsl), but not brummer (adipose triglyceride lipase homologue), to the surface of the LDs when co-overexpressed in Drosophila S2 cells under a condition that induces lipolysis.
The Drosophila larval fat body (human adipose tissue and liver equivalent) stores lipids in large LDs and releases them during later development or during starvation conditions. We employed various *Pex* mutant and fat body-specific *Pex* knockdown (*Pex* FKD) flies to examine roles Peroxins have in lipid storage *in vivo*. When wild-type (WT) and *Pex* FKD (*Pex1*, *Pex13*, and *Pex14*) were fed on holidic food (low fat), they remained relatively stable and exhibited similar survival rates. When raised on holidic+lard food (high fat), the survival rates of both WT and *Pex1* FKD larvae remained similar to the previous feeding condition while *Pex13* and *Pex14* FKD displayed a reduction in their survival rates suggesting lipotoxic effects due to an abnormal increase in systemic lipid levels. Compared to the WT larvae, *Pex13*, *Pex14*, and *Pex16* FKD showed reduced brain size while *Pex11* and *Pex19* did not seem to be affected.

Compared to the WT larvae, only *Pex13* and *Pex14* mutants displayed reduced fat storage in the fat body indicated by reductions in size and quantity of LDs while *Pex1* and *Pex5* mutants seemed to display increased fat storage. Intriguingly, the dsRNA-mediated KD of *Pex16* in S2 cells dramatically reduced *Pex14* mRNA expression suggesting co-regulation of the two *Pex* genes. Overall, the evidence seems to support the unique roles of both Pex13 and Pex14 in promoting lipid storage in the fat body of *Drosophila melanogaster*. Moreover, these roles are independent of peroxisomes as other *Pex* FKD and mutants that also similarly affect peroxisome functions were seemingly unaffected.

677A Identifying the minimal sequence that enables protein trafficking to the B-body, a novel nuclear domain *Shania Kalladanthyil*, Miranda Adams, Kaveh Kiani, Luis Rodriguez Kennesaw State University

Nuclear domains (ND) are the areas inside the cell nucleus with sharp boundaries and specific protein composition. Mechanisms that control the existence of various nuclear domains are not fully understood. The B-body is a prominent ND that was recently discovered in developing Drosophila flight muscles. We used B-body's resident protein Bruno 1 (Bru) as a model to dissect out the minimal protein sequence that is required to enable trafficking to this ND. Our study was facilitated by the relatively simple domain structure of Bru, which consists of one paired and one single RNA-recognizing motifs (i.e., RRM1/2 and RRM3), separated by two intrinsically disordered regions (IDR1 and IDR2). RNA-binding ability is critical for B-body affinity since ectopic RNAse treatment releases Bru from B-bodies in muscle cryosections. Using transgenic flies expressing GFP-tagged Bru mutants, we determined that B-body affinity depends on the functionality of RRM1/2, but not RRM3. Next, a series of truncation mutants were deployed to probe for the minimal sequence required for B-body accumulation. Neither the N-terminal IDR1 nor RRM1/2 alone nor in combination could accumulate in the B-body. However, RRM1/2 combined with the C-terminal IDR2 retained B-body affinity. Our study reaffirms the importance of domain combinations for the proper ND trafficking while highlighting the surprising selectivity of IDRs in this process. Future studies should determine if the affinity to B-body can be decoupled from the nuclear functions of the Bru protein.

678B Characterization of the physical and functional connection between CNK and Misshapen *Eloïse Duramé*[1,2], Caroline Baril[1,2], Malha Sahmi[1,2], Marc Therrien[1,2] 1) Université de Montréal; 2) Institut de Recherche en Immunologie et Cancérologie (IRIC)

Connector eNhancer of KSR (CNK) is an evolutionarily conserved scaffold protein acting as a key regulator of the RAS-MAPK signaling pathway in Drosophila. To discover new functions of CNK, we captured the proximal interactome of CNK in Drosophila S2 cells using an improved version of the BioID technique called miniTurboID. This identified components of the RAS-MAPK pathway known to interact with CNK such as KSR, MEK, RAF and HYP. In addition, we identified the Ste20-related kinase Misshapen (MSN), which is involved in several biological events related to cell migration and planar cell polarity. To decipher the connection between CNK and MSN, we first tested whether CNK and MSN could physically interact in S2 cells. Co-immunoprecipitation experiments demonstrated their ability to associate in S2 cells. This interaction depends on the N-terminal part of CNK (containing the SAM, CRIC and PDZ domains) and the C-terminal part of MSN (containing the CNH domain). In another set of experiments, we obtained the common proximal interactors of CNK and MSN in S2 cells using miniTurboID. Strikingly, we identified 72 common interactors to CNK and MSN, which are largely associated to vesicular trafficking, cytoskeletal organization, cell polarity and migration. Finally, we used the *UAS/GAL4* system to look for a genetic link between CNK and MSN. We found that knockdown of MSN during eye development had no effect on the level of phosphorylated MAPK and photoreceptor differentiation in third instar larva suggesting that the function of MSN is not related to RAS-MAPK signalling. Interestingly, we found that depletion of MSN during wing development produced wing blisters in ~40% adult Drosophila analyzed while depletion of CNK had no effect. However, co-depletion of MSN and CNK produced wing blisters in ~70% of adult flies, thus providing the first evidence of a genetic link between CNK and MSN. Blister formation in adult Drosophila wing generally results from improper apposition/adhesion of the wing epithelial bilayer. Imbalance in integrin signalling, vesicular trafficking and cytoskeletal organization have been linked to blister formation. We are now investigating the role of CNK and MSN in those biological events. Our findings suggest that CNK is not exclusively devoted to RAS-MAPK signaling, but that it might work in other signaling events that play critical roles during vesicular trafficking, cytoskeletal organization and cell migration.

679C Septins are necessary for detachment and protrusion formation in border cell migration *Allison Gabbert*, James Mondo, Joseph Campanale, Denise Montell UC Santa Barbara

Collective cell migration is crucial for development and the preferred mode of migration by metastatic tumors, but much about it is unknown. The border cell cluster in the *Drosophila* ovary is an ideal model for collective cell migration, as border cells migrate on and between nurse cells. The cytoskeleton is a critical regulator of cell migration. Septins, now considered the fourth cytoskeletal element, remain unexplored in collective systems. The objective of this study is to understand the functions and necessity of septins in border cell migration. Using RNAi, mutants, and over-expression, we investigated the impact of septins on border cell migration and cluster morphology through fixed and live imaging. We used high-resolution Airyscan imaging paired with Tissue Cartography to generate 3D models of the surface of the

cluster. We found that knocking down any of the five *Drosophila* septins significantly impacted border cell migration and cluster morphology through detachment failure, failure to form stable forward-directed protrusions, and a loose blebby morphology. Overexpressing septins also dramatically impacted migration, with an inverse effect on morphology. As septin subunits function by forming higher order structures with each other, we investigated if septin monomers interacted. Clonal knockdown of Septin 1 (Sep1) or Septin 2 (Sep2) led to a significant loss of Peanut (Pnut, or Sep3) in the follicle and border cells. Similarly, knockdown of Sep1 or Pnut caused a loss of Sep2. These findings suggest that Sep1, Sep2, and Pnut form structures with each other in the follicle and border cells independently of Sep4 and Sep5, which have no impact on Pnut or Sep2 expression. To uncover the mechanistic role of septins in border cell migration, we tested candidates that may interact with septins. We observed co-localization between septins and nonmuscle myosin II in fixed imaging, and then explored further through live imaging. Amazingly, dynamic myosin flashes completely co-localized with dynamic septin expression. This suggests an interaction between septins and myosin. For example, septins may act as a scaffold for myosin, recruiting it and stabilizing protrusions. In conclusion, we found that septins are necessary for the detachment of the border cell cluster and for protrusion formation, while too much septin induces an excess of curvature and prevents migration. Sep1, Sep2, and Pnut interact to form structures and may interact with myosin.

680A Nuclear lamins promote collective cell migration and coordinate protrusion dynamics *Lauren Penfield*, Denise Montell University of California, Santa Barbara

Cells migrate collectively during embryonic development and cancer metastasis. Determining how groups of cells detect and respond to confined tissue environments *in vivo* is a major goal. The nucleus, which is generally the stiffest organelle, deforms and sometimes ruptures to move through confined environments. While *in vitro,* the stiffness of the nucleus impedes movement of single cells through confined spaces*,* the effects of nuclear stiffness *in vivo* are unknown. Further, the roles of nuclei in collective cell migration are not clear. Here, we use border cell migration in the fly ovary as an *in vivo* model to investigate the effects of confined migration on nuclei. We found severe yet transient nuclear deformations occur, particularly in the leading cell, as border cells squeeze through tiny crevices between germline cells, termed nurse cells. These spaces are narrower than even a single border cell nucleus. Leading cells extend protrusions between nurse cells, which may pry open spaces to allow the cluster to traverse. The nucleus in the leading cell deforms as it moves through the neck of the protrusion and restores a more circular shape as the protrusion widens. In contrast, nuclei in the following cells had less dynamic movement and shape changes. These data suggest that nuclei in leading cells may widen protrusions to expand the size of the migration path and allow the cluster to move forward. To experimentally test how nuclei contribute to border cell migration, we investigated nuclear lamins, proteins that assemble into intermediate filaments to structurally support the nucleus. Depletion of the Drosophila B-type lamin, Lam, from the outer motile border cells, but not the inner, nonmotile polar cells, impeded border cell migration. Surprisingly, perturbations of the Drosophila A-type lamin, LamC, did not impair migration. While wildtype border cell clusters normally have one leading protrusion as they migrate, border cells depleted of B-type lamin had multiple, short-lived protrusions, resulting in unproductive cluster movement and failure to progress down the migration path. Further, border cell nuclei depleted of B-type lamins were smaller, formed blebs, and underwent rupture. Together, these data indicate that B-type lamins maintain nuclear structure, and this is required to stabilize the leading protrusion and promote collective cell migration through confined spaces.

681B Investigating the initiation of collective cell migration in the *Drosophila* follicular epithelium *Sierra Schwabach*, Sally Horne-Badovinac University 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 follicular epithelial cells in the Drosophila egg chamber, which helps to create the elliptical shape of the mature egg. During the formation of a new egg chamber, follicle cells initiate a collective migration perpendicular to the anterior-posterior (AP) axis. In many models of collective migration, a subset of the cells contact free space which can act as an external polarizing cue for the cells to start migrating. In contrast, the follicle cells form a topologically closed epithelium that lacks a free edge. As a result, the direction of follicle cell migration in each egg chamber is stochastic and can occur either clockwise or counterclockwise with respect to the AP axis. Follicle cell migration depends on a type of planar polarity, in which the atypical cadherin Fat2 localizes to the trailing edge of each cell and signals to the cell behind to promote migration. Interestingly, we know that the maintenance of Fat2's planar polarized localization also requires migration of the tissue, which is driven by SCAR/WAVE-dependent protrusions at the leading edge of each follicle cell. This leads to our model that polarized Fat2 signaling and migration promote one another in a self-reinforcing, mutually dependent feedback loop. While we have a grasp on the mechanisms that promote follicle cell migration once it has started, it is unknown how follicle cells initiate collective migration without the existence of an external polarizing cue. We hypothesize that it is the interplay between motility and polarized Fat2 signaling that allows the tissue to self-organize and initiate collective migration. We have taken two different approaches to address this hypothesis. The first includes improving upon current live imaging methods to watch migration initiation unfold in its natural context. The second approach utilizes the GAL4/UAS system to delay migration

initiation until the egg chambers are older and easier to image. Preliminary results from both methods will be discussed at the poster.

682C Control of Crag's localization and activity in the polarized deposition of basement membrane proteins in epithelial cells. *Hemin Shah*[1], Alex Hoover[1], Megan Gladwin[2], Trudi Schüpbach[2], Olivier Devergne[1] 1) Department of Biological Sciences, Northern Illinois University, DeKalb, IL; 2) Department of Molecular Biology, Princeton University, Princeton, NJ

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 our 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, it assumes a polarized localization and accumulates apically and laterally through yet unknown mechanisms. Importantly, specific localization of the GEF Crag is thought to be critical for the localized activation of Rab10. Thus, to understand how Crag controls the polarized deposition of BM proteins, it is critical 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.

683A Basement membrane repair dynamics in the *Drosophila* midgut *Aubrie Stricker*, Kimberly LaFever, Katherine Peebles, M. Shane Hutson, Andrea Page-McCaw Vanderbilt University, Nashville, TN

Basement membranes are the oldest, most conserved forms of extracellular matrix and serve to separate tissue layers, provide mechanical support, direct signals to neighboring cells, and insulate tissues from signals. Further, basement membranes are subject to mechanical damage and require dynamic repair mechanisms. Faulty basement membrane repair mechanisms can aid in the progression of diseases such as asthma and diabetes, and diseases of the basement membrane itself, including Alport's syndrome and Goodpasture's syndrome. Therefore, understanding how basement membranes repair will be vital to treating these conditions. Our work utilizes the *Drosophila* midgut basement membrane to probe repair dynamics. In *Drosophila*, all major basement membrane components have been conserved but with less redundancy than mammals. Our lab has developed an assay to reproducibly damage the basement membrane and study the repair process. Previously we reported that many aspects of basement membrane repair are shared during homeostasis. Thus, it is unclear whether basement membrane damage is actively detected, or instead, passively repaired by homeostatic mechanisms.

Our recent data suggests basement membrane damage is actively detected. Following damage, there is an increase in the number of enteroendocrine (EE) cells, the major secretory cell type in the gut, and we find that guts without EE cells cannot repair the basement membrane. Additionally, the EE cells are a significant contributor of collagen IV needed to repair damage. Importantly, EE precursor cells express a mechanosensory stretch-activated ion channel, Piezo, raising the possibility that a change in stiffness of damaged basement membranes signals the initiation of repair. Excitingly, Piezo knockout flies are able to maintain basement membrane homeostasis in the adult fly but cannot repair it after damage. This is our first evidence that there is a unique mechanism to detect basement membrane damage and initiate repair. However, to our surprise, Piezo knockout flies also showed an increase in EE cells following damage, suggesting that EE cells are regulated independently of Piezo. This result suggests that Piezo and EE cells function independently to repair basement membrane, raising the question of how Piezo functions in basement membrane repair.

684B The mystery of the P*eroxidasin* mutant: why does this catalytically dead *Drosophila* mutant survive?

Katherine Peebles[1], Kimberly LaFever[1], Gautam Bhave[1,2], Andrea Page-McCaw[1] 1) Vanderbilt University; 2) Vanderbilt University Medical Center

The basement membrane is a sheet-like extracellular matrix that underlies epithelia and surrounds muscles. In the

gut of *Drosophila*, the stiff basement membrane surrounds the muscles used in peristalsis to keep them flat and smooth. Collagen IV is one of the main components of the basement membrane, where it adds structure and stiffness. The *Peroxidasin* (*Pxn*) gene encodes an enzyme that crosslinks collagen IV at the NC1 domain. This crosslinking supports basement membrane stability and contributes to its stiffness. Hypomorphic P-element mutations of *Pxn* exist in *Drosophila* that survive to the larval stages with decreased viability to adulthood, but no null mutation has been reported. We expected a null mutation to be lethal at the end of embryogenesis, when collagen IV mutants die. Using CRISPR, we created a mutant (Pxn^{11}) that deletes a portion of the catalytic domain, eliminating its activity. Further, this deletion also caused a frameshift mutation that inserted a stop codon soon after the deletion. Contrary to our expectations, about twenty percent of expected homozygotes survive and live an apparently normal lifespan. These homozygotes exhibit muscle defects in the gut consistent with loss of stiffness in the basement membrane. A few hypotheses that could account for their viability will be discussed.

685C The role of ZP domain proteins in controlling corneal lens architecture *Neha Ghosh*, Hongsu Wang, Jessica Treisman Skirball Institute of Biomolecular Medicine, NYU School of Medicine

The shape of organs can be critical to their function; for example, changes in the curvature of the human cornea result in visual disorders such as myopia, hypermetropia, astigmatism or keratoconus. A simpler model system like *Drosophila* may provide insights into the development of curved refractive structures such as the cornea. Each ommatidium of the *Drosophila* eye contains a biconvex corneal lens that focuses light on the underlying photoreceptor cells. The central part of the corneal lens is thought to be secreted by the underlying cone and primary pigment cells, while the peripheral portion is attached to the secondary and tertiary pigment cells. Here we show that loss of the zinc-finger containing transcription factor Blimp-1 specifically from the secondary and tertiary pigment cells causes flattening of the external corneal lens surface, dramatically reducing its refractive power. One possible mechanism for this effect of Blimp-1 would be a change in the pattern of attachments of the corneal lens to the peripheral pigment cells. The fly corneal lens is a cuticular structure containing the polysaccharide chitin, and the morphology of other chitinous structures such as denticles and tracheal tubes has been shown to depend on the localization of specific zona pellucida (ZP) domain containing proteins, which attach the apical extracellular matrix to the plasma membrane. In a transcriptomic study of the effects of *Blimp-1* knockdown in the mid-pupal retina, we observed changes in the expression levels of several genes encoding ZP domain proteins; *dusky-like* (*dyl*), *CG10005* and *cypher* (*cyr*) were downregulated, while *neyo*, *piopio* and *quasimodo* were upregulated. We find that loss of *dyl* or *cyr* also causes flattening of the outer surface of the corneal lens. These observations suggest that Blimp-1 may influence corneal lens architecture in part by ensuring the appropriate distribution of the ZP proteins that attach its periphery to the secondary and tertiary pigment cells.

686A Neural IgCAMs at work in epithelia: phylogeny and function *Colleen Maillee*, Dan Bergstralh University of Rochester

Epithelial cells are linked together by adherens junctions, which are largely responsible for cell-cell adhesion, and occluding junctions, which form a barrier to paracellular diffusion. Our published results show that another epithelial adhesion module, comprised of Ig-domain adhesion proteins – Neuroglian/L1CAM, Fasciclin II/NCAM, and a three Ig-domain protein, either Fas3 (insects) or potentially CADM1 (vertebrates) – helps to maintain epithelial integrity during proliferation by ensuring that the tissue grows as a monolayer. These factors are not only found in proliferating epithelia, where they can localize along the length of the cell-cell border, but also in the vertebrate excitatory synapse and *Drosophila* neuromuscular junction. Furthermore, they have been extensively studied in both flies and mammals for their role in axon guidance. We want to know how they work. L1CAM is evolutionarily conserved at the sequence level, and the function of its cytoplasmic domain is well studied. Remarkably, whereas the extracellular C-terminal structures for NCam/Fas2 and CADM1/Fas3 are similar, their cytoplasmic N-termini are not. These findings suggest conservation of function without conservation of sequence. We are using phylogenetic analysis, structural prediction algorithms, and *Drosophila* genetics to study this problem.

687B Fatty acid trafficking during Drosophila oogenesis *Roger White*, Michael Welte University of Rochester, Biology department, Rochester, NY

Lipid droplets (LDs) are ubiquitous fat storage organelles with essential roles in lipid metabolism, including storage and trafficking of fatty acids. During Stages 9-10B of oogenesis, Drosophila egg chambers accumulate hundreds of thousands of LDs containing triacylglycerol (TAG) and sterol esters. Most of the fatty acids (FAs) in these stored lipids ultimately come from lipophorin (LPP) particles circulating in the hemolymph. Previous work has led to the following working model for how these FAs are funneled into LDs: LPP particles dock on the nurse cell plasma membrane via lipophorin receptors and are broken down by extracellular lipases. The liberated FAs are then taken up via FA transporters, activated by acyl-CoA synthetases and converted to TAG by DGAT1/Midway. The long-term goal of this project is to critically test this model and identify the molecular players and regulatory steps involved. To this end, we have developed a protocol to monitor FA trafficking during oogenesis using fluorescently labeled fatty acid (FLFA). When flies are fed food

supplemented with various FLFAs, FLFA accumulates in nurse cell LDs. Preliminary evidence suggests that when flies are starved prior to FLFA feeding FLFAs do not enrich in LDs, but appear to accumulate in mitochondria, suggesting that egg chambers direct FLFA to different intracellular locations according to the physiological state of the animal. When isolated egg chambers are incubated in FLFA containing media, we observe incorporation in LDs already after 15 mins, providing an inroad for observing FA trafficking to nascent LDs. In mutants for *midway,* no LDs form and egg chambers arrest at Stage 9. Using FLFA supplementation, we found that in the mutant egg chambers FLFA is still taken up, but accumulates in mitochondria, even for well-fed flies. In addition, nurse cell mitochondria take on an abnormal morphology. We hypothesize that when incoming FAs are not properly sequestered in LDs, they inappropriately accumulate in mitochondria, causing dysfunction. Intriguingly, reducing the dosage of *Eip75B*, a Drosophila PPAR homolog, allows *midway* mutant egg chambers to develop further, implicating dysregulated lipid signaling in the developmental arrest. In summary, our results suggest that during oogenesis LDs are critical for regulating FA trafficking and usage and are key for mitigating lipotoxicity.

688V Rap1 acts via Canoe and Rho1 to control the adhesion and cytoskeletal rearrangements that drive rapid wound repair *Katheryn Rothenberg*, Rodrigo Fernandez-Gonzalez University 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, which allows for genetic, pharmacological, and biophysical manipulations. 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 accumulation of cell-cell adhesion components. However, the mechanisms that coordinate the adhesive and cytoskeletal rearrangements required for rapid wound closure are unclear. The small GTPase Rap1 is a mechanosensitive molecular switch that promotes cytoskeletal polarization and regulates cell adhesion turnover in other systems. Using quantitative time-lapse microscopy of fluorescently tagged proteins, we found that Rap1, the Rap1 effector Canoe/Afadin, and E-cadherin were simultaneously depleted from the wound edge and accumulated at TCJs. Reducing Rap1 activity by overexpressing a dominant-negative Rap1 (Rap1DN) slowed wound repair by 45%. The slower wound closure was accompanied by defective actomyosin polarization to the wound edge and a 25% loss in E-cadherin accumulation at TCJs compared to a 25% increase in controls. Together, our data indicate that Rap1 is necessary for rearranging cell-cell adhesions and creating a stable force-generating actomyosin cable to drive rapid wound closure. Consistent with this model, Canoe knock-down led to 44% slower wound repair and 32% lower E-cadherin fluorescence at TCJs. These results suggest that Rap1 signals through Canoe to reinforce TCJs during wound closure. To understand how Rap1 may be affecting assembly of the actomyosin cable, we measured Rho1 activity in Rap1DN embryos and found that Rho1 activity was reduced by 72% in Rap1DN embryos, which is accompanied by a 54% reduction in tension at the wound edge. However, Canoe knock-down does not affect Rho1 activity. Our data support a model in which Rap1 simultaneously drives actomyosin cable assembly via activation of Rho1 and reinforced adhesion at TCJs via Canoe/Afadin.

689V Cell wound repair requires the coordinated action of linear and branched actin nucleation factors *Justin Hui*, Mitsutoshi Nakamura, Susan Parkhurst Basic Sciences Division, Fred Hutchinson Cancer Research Center, Seattle, WA

During cellular wound repair, actin and other cytoskeletal proteins act in concert to coordinate wound closure in a timely fashion. A critical step is to form an actomyosin ring that contracts to close the wound. This process is dynamically regulated in part by Rho family GTPases, which through their different effectors can regulate both linear and branched actin nucleators to facilitate cytoskeletal dynamics. We have previously shown that knockdown of the formin Diaphanous, a linear actin nucleator and downstream effector of Rho, results in prolonged wound closure and the inability to form a proper actomyosin ring. However, when all linear nucleation factors are removed, some actin organization at the wound persists. We investigated the contribution of branched nucleation factors to cell wound repair. We show that the Wiskott-Aldrich Syndrome (WAS) protein family members (WASp, Wash, and SCAR), and their cofactor Arp2/3, play non-redundant roles in regulating actin organization and architecture of the contractile actomyosin ring. Notably, we observe stark differences in the spatial and temporal recruitment patterns to wounds among the WAS proteins during the wound repair process. In particular, WASp is recruited early, SCAR recruitment peaks late, and Wash is present throughout the process. Further, individual knockdown of the WAS proteins resulted in different actomyosin ring architectures, including differences in mesh density and filament orientation. Dynamically, WASp knockdown exhibits slower contraction whereas SCAR contracts faster and Wash contracts similarly to wildtype. Interestingly, in the absence of branched actin, wounds exhibited an abundance of elongated linear filaments that colocalize with Diaphanous, as well as other unique actin structures, which colocalize with Myosin. When both linear and branched nucleation factors are simultaneously knocked down, we observe a significant decrease of these elongated filaments and the absence of previously seen actin structures. We also inhibited myosin activity in an Arp3 knockdown and observed a surprising wound repair phenotype consisting of spiraling linear filaments and excessive actin bundling. Our results

emphasize the strong requirement for balance and crosstalk among linear and branched actin nucleators, as well as myosin, to facilitate proper actin filament architecture, organization, and dynamic contractile closure of the actomyosin ring during cell wound repair.

690V Molecular regulation of centrosome stability *Ana Pimenta-Marques*, Tânia Perestrelo, Patrícia Rodrigues, Paulo Duarte, Mariana Faria, Mónica Bettencourt-Dias Instituto Gulbenkian de Ciência

An important feature for cell homeostasis is how structures such as different organelles are maintained in the cell. This is particularly relevant for organelles whose number and function are under a tight control, as deregulation of these properties has critical implications for the cell. This is the case of the centrosome. The centrosome is the main microtubule-organizing center of eukaryotic cells. This organelle is composed of two centrioles, surrounded by a multiprotein matrix called the pericentriolar
material (PCM) which is critical for microtubule nucleation.
Centrosomes are thought to be very stable and can persist over long periods of time. However, these structures can disappear in certain developmental stages and their numbers and structure can be deregulated in several diseases. Moreover, some centrosome components are quite dynamic. While a large body of knowledge has been produced regarding the biogenesis of these structures, little is known about how they are maintained. Our previous work showed that both POLO kinase and PCM are critical players in a centriole maintenance program which is shut off in the female germ-line to allow fertility. We have now investigated the players involved in this program, at the level of the centriole. By studying centrosome stability in the female germline and in Drosophila cultured cells, we found that several centriole components play a role, amongst them the centriolar wall protein ANA1 has a very strong phenotype associated with the centrosome maintenance program. We then further show that the stability conferred by Polo and the PCM to centrosomes is dependent on ANA1, and that ectopic targeting of ANA1 can prevent centrosome loss in oogenesis. Our work suggests that the PCM functions as a concentrator of components, in particular ANA1, which are required for centriole maintenance. We will discuss our data and how it provides a deeper understanding on the molecular mechanisms by which centriole stability is regulated.

691V Evolutionary Diversification of *Drosophila* Arp2 for Specialized Actin Branching Sarah Tomlin[2], Tristan Spain[1], Harmit Malik[2,3], *Courtney Schroeder*[1] 1) UT Southwestern Medical Center; 2) Fred Hutchinson Cancer Research Center; 3) Howard Hughes Medical Institute

The actin cytoskeleton, which is composed of force-generating polymers, often forms branched networks that are critical in many fundamental cellular processes, including cell motility, cell division and vesical movement. Branched actin networks are generated by the Arp2/3 complex, a 7-membered protein complex including actin-related proteins (Arps) 2 and 3. Similar to actin and most Arps, Arp2 is evolutionarily ancient among eukaryotes and under stringent sequence conservation, yet we surprisingly discovered two clade-specific gene duplications of Arp2 in *Drosophila*: *Arp2D* in the *obscura* clade and *Arp2D2* in the *montium* clade. Our targeted sequencing and phylogenetic analyses of *Arp2D* and *Arp2D2* show these duplicates have evolved independently and arose ~14 million years ago at the origin of their respective clades. The two duplicates exhibit distinct sequence diversification from canonical Arp2, and unlike the ubiquitously expressed Arp2, both duplicates are testis-enriched in expression. Why would evolution recurrently select for a divergent Arp2 for potential roles in the male germline? To elucidate the function of these duplicates, we investigated whether both duplicates can polymerize branched actin networks similar to canonical Arp2. We replaced canonical *Arp2* in *D. melanogaster* with *D. pseudoobscura Arp2D* or *D. auraria Arp2D2* and found they can both rescue the *Arp2* knockout lethality phenotype, suggesting the duplicates can indeed polymerize branched actin networks despite their sequence divergence. Cytological analyses further confirmed the two duplicates localize to branched actin networks. Surprisingly, however, *Arp2D*-expressing flies have significantly reduced fertility unlike *Arp2D2*-expressing flies. Our findings suggest that canonical Arp2's roles can largely be partitioned between somatic versus germline roles, and we hypothesize that while both Arp2 gene duplicates can polymerize branched actin in somatic cells, Arp2D fails to generate proper actin networks for germ cell development. We are currently investigating the integrity of germline actin in the transgenic flies and exploring how Arp2D's sequence divergence has perhaps led to specialized actin polymerization for germline biology.

692V Centrosome-induced membrane infolding linked to Rac pathway and Arp2/3 network recruitment during actin cap formation in the *Drosophila* embryo *Rebecca Tam*, Nicolas Vergara Ruiz, Tony Harris University of Toronto

The cell cortex is a dynamic network of proteins that allows cells to change shape during processes like migration and division. Local activation of the Rac-GEF pathway leads to the assembly of Arp2/3 networks which change the cell cortex. Moreover, centrosomes influence the cortex during cell migration and division. How centrosomes activate the assembly of actin networks is not well understood. During early *Drosophila* embryogenesis, an actin cap forms above the nucleus and the growth of the cap creates a dome-like structure that houses the mitotic apparatus. The actin cap forms below the plasma membrane and requires centrosomes and the Rac-GEF, Sponge. What remains unclear is how centrosomes promote the local recruitment of the Rac-GEF pathway. My current research shows that the plasma

membrane forms apical folds and sub-apical tubules above the centrosomes. Moreover, Spg, Rac-GTP, Scar, and Arp2/3 localized to these membrane structures above the centrosomes where nascent actin caps form. To closely investigate the relationship between centrosomes, the plasma membrane, and the Rac-GEF pathway, we inhibited cap expansion by depleting Arp3 using RNAi. Membrane folds accumulated excessively above expected centrosomes and sub-apical membrane tubules persisted. In addition, the Rac-GEF pathway proteins showed higher levels of buildup above expected centrosomes compared to the control. Notably, the plasma membrane and the Rac-GEF pathway proteins had a more focused relationship with the centrosomes in Arp3 RNAi, suggesting Arp2/3 network growth normally spreads the components away from the sites of initial cap induction. To see how the centrosomes influence the plasma membrane and the Rac-GEF pathway localization, centrosomin (Cnn) RNAi was used. In Cnn RNAi, the membrane was disorganized, with membrane folds lacking specific association with the centrosomes and sub-apical tubules being less numerous. Furthermore, the accumulation of the Rac-GEF pathway was diminished in Cnn RNAi. Together, these results suggest that the centrosome promotes the localization of the Rac-GEF pathway through a mechanism linked to local plasma membrane infolding.

693V Early endosomal Rab21 in enterocytes contributes to gut tissue maintenance *Sonya Nassari*, Steve Jean Université de Sherbrooke

Membrane trafficking describes the vesicular transport of proteins and macromolecules by cells. Trafficking defects impair signaling events and have dramatic consequences on cell homeostasis. Enterocytes (ECs), intestinal differentiated cells, are the main cell type of the gut epithelium. Given their absorptive functions, they display a high membrane trafficking flux. Notably, defects in endocytic pathways can affect ECs function and lead to intestinal bowel diseases. However, how does trafficking regulate intestinal tissue maintenance is poorly understood. Rab21 is a small GTPase involved in early endosomal trafficking. It was first identified in human intestinal cells and is expressed in ECs. Importantly, Rab21 levels are drastically decreased in ECs upon intestinal inflammation.
Using the Drosophila intestine as an *in vivo* model system, we investigated EC-specific functions for Rab21 in gut tissue maintenance. We monitored and assessed Rab21 loss of function in ECs of adult flies. Rab21 depleted guts showed severe intestinal morphology abnormalities. Furthermore, cellular equilibrium was affected, and a gain in mitotic cells, associated with increased cell death was observed. Interestingly, we found that an increased level of the IL-6 like cytokine, Upd3, in ECs was responsible for overproliferation, through activation of the JAK/STAT pathway mediated via yki activity and apoptosis.
To further characterize Rab21 functions, we performed a TMT-based quantitative proteomic analysis for Rab21 depleted guts and identified 101 proteins significantly modulated. We investigated the functional link between Rab21 and two overabundant proteins: ApoLpp, an apolipoprotein involved in diacylglycerol mobilization and Tret1-1, a trehalose transporter. We observed that Rab21 silencing in EC affects lipid content in the gut, as well as in the fat body, while circulating lipids did not appear affected, highlighting an unappreciated lipid-related function for Rab21. Furthermore, we demonstrated that loss of Rab21 in EC impaired trehalose levels in hemolymph, suggesting the involvement of Rab21 in the regulation of trehalose distribution, likely via specific trafficking of the trehalose transporter.
Altogether, our data indicate that EC Rab21 plays an important role in intestinal tissue maintenance and functions. Although Rab21 specific roles on lipids and trehalose regulation remain to be defined, we show that Rab21 contributes to ECs survival and their correct function.

695V Implications of Class II PI3K Variants and Mtm phosphatase during Autophagic Lysosome Reformation *Ilva Cabrera*, Jean-Francois Groulx, Amy Kiger University of California, San Diego La Jolla, CA

Macroautophagy (autophagy) is a trafficking pathway that relies on delivery of membrane-bound cytoplasmic contents for degradation by the lysosome, referred to as the autolysosome. The lysosome is a very important component in autophagy regulation as it must be able to accommodate the convergence of cargos from both autophagy and endocytosis pathways, as well as coordinate autolysosome maturation with changing levels of basal versus stress-induced autophagy. In order for to replenish the lysosomes during prolonged starvation-induced autophagy, one-way new lysosomes can be made is through the autophagic lysosome reformation (ALR) pathway. Tubule-like projections from lysosomes pinch-off to form proto-lysosomes, eventually maturing into new functional lysosomes. We identified two phosphoinositide lipid regulators, class II PI3-kinase (PI3KC2) and Myotubularin PI3-phosphatase (Mtm), that act in a shared autolysosome inhibitory pathway to maintain basal levels of autophagy in the fat body of fed Drosophila larvae. Strikingly, we discovered that wildtype flies also co-express a truncated, noncatalytic PI3KC2 splice variant (PI3KC2-short) that, converse to PI3KC2 and Mtm, is required for autolysosome maturation through repression of their catalytic activities. We have shown that defects in autolysosome maturation correlate with aspects of (ALR). Our work addresses the importance of coordinated activity between specific phosphoinositide kinase and phosphatase activities. In addition, this study reveals a novel role for their joint regulation by a noncatalytic splice variant to respond to changing homeostatic demands in membrane flux.

696V A new shape, a new fate: uncovering how mitochondria regulate germline stem cell differentiation *Vernon Monteiro*, Thomas Hurd Department of Molecular Genetics, University of Toronto, Toronto, Ontario, Canada

Stem cell differentiation is essential for development and tissue homeostasis. Often differentiation is accompanied by a rewiring of organelles and alterations of the metabolome. However, how mitochondrial dynamics contributes to, and influences differentiation *in vivo* remains poorly understood. Using the *Drosophila* germline as a model system, we find that remodelling of the inner mitochondrial membrane is coupled to germline stem cell (GSC) differentiation. During the early stages of differentiation, mitochondrial Complex V is essential to drive the remodelling of cristae and in its absence the immediate progeny fail to survive. The failure to remodel mitochondria during early germ cell differentiation results in activation the Integrated Stress Response through the ER membrane kinase, PERK. PERK does so by detecting alterations in the ER membrane. Furthermore, the loss of Complex V and the failure to remodel mitochondria, induces the formation of lipid droplets that is partially mediated through PERK activity. Taken together, our data suggest that the loss of inner mitochondrial remodelling during early germline stem cell differentiation destabilizes the ER membrane thereby activating PERK and inducing cell death.

697V Cell-extracellular matrix adhesion is necessary for rapid embryonic wound closure *Michelle Ly*[1,2,3], Katheryn Rothenberg[1,2], Clara Schimmer[1,2], Raymond Hawkins[1,2], Rodrigo Fernandez-Gonzalez[1,2,3,4,5] 1) Institute of Biomedical Engineering, University of Toronto, Canada; 2) Translational Biology and Engineering Program, Ted Rogers Centre for Heart Research, University of Toronto, Canada; 3) Collaborative Specialization in Developmental Biology, University of Toronto, Canada; 4) Department of Cell and Systems Biology, University of Toronto, Canada; 5) Developmental and Stem Cell Biology Program, The Hospital for Sick Children, Canada

Embryos repair wounds rapidly, with no inflammation or scarring. In embryos, wound repair is driven by the collective movement of the cells around the wound to seal the lesion. Embryonic wound healing is associated with reorganization of the actomyosin cytoskeleton and cell-cell adhesive structures in the cells adjacent to the wound. Cell migration often involves adhesion to and remodeling of the extracellular matrix (ECM) through integrin-based adhesions. However, the role of integrin-based adhesion in embryonic wound closure has not been investigated. To establish the role of cell-ECM adhesion in embryonic wound repair, we imaged and wounded the epidermis of embryos mid-way through development (12-14 hours) using a pulsed ultraviolet laser mounted on a spinning disk confocal microscope. Using embryos expressing fluorescently labeled talin, we found that upon wounding, the cells adjacent to the wound polarized talin to the wound edge, suggesting increased cell-ECM interactions at the wound margin. To determine the impact of cell-ECM adhesion on embryonic wound closure, we injected embryos with RGDS, a peptide that binds integrins and inhibits ECM binding. RGDS treatment delayed wound closure by 43% with respect to controls. Imaging of fluorescently labeled myosin II revealed that the delay in wound healing occurred with no significant effects on the myosin rearrangements associated with wound repair. We obtained similar results when we knocked down integrins using RNA interference against the β integrin subunit: wound closure displayed a 54% delay compared to controls with no changes in myosin dynamics around the wound. However, we found a 71% decrease in actin accumulation around the wound edge relative to controls. We also noticed a defect in the E-cadherin reorganization necessary for actomyosin remodelling during wound healing. In control embryos, E-cadherin accumulated at former tricellular junctions at the wound margin, displaying a 29% increase in tricellular junction E-cadherin fluorescence 15 minutes after wounding. In contrast, RGDS treatment resulted in a 13% decrease in tricellular junction E-cadherin at the same time point. We obtained similar results in integrin RNAi embryos, which displayed a 20% decrease in tricellular junction E-cadherin. Together, our results suggest that cell-ECM adhesion contributes to wound repair and reveal a previously unrecognized interplay between cell-cell and cell-ECM adhesion in the collective cell movements that drive embryonic wound closure.

698V Cell extrusion during starvation-induced intestinal shrinkage *Aparna Sherlekar Banerjee*, Elsa Su, Samantha Thomas, Keerthana Yellapragada, Lucy Erin O'Brien Stanford University School of Medicine

The adult *Drosophila* midgut shrinks as a result of nutrient deprivation. Shrinkage is in part due to reduction in enterocyte cell volume and cell loss by enterocyte cell extrusion - there was two-fold increase in extrusions upon fasting. At steady-state most cells activated executioner caspases before they extruded. Inhibition of caspase activation blocked extrusions at steady-state. However, in fasted guts, ~70% of extruded cells were negative for cleaved caspase. TOR signaling is a conserved pathway that senses and responds to nutrient signals. Paradoxically, TOR signaling activation by inhibiting Tsc2 or Rheb overexpression increased cell extrusions in the midgut. Tsc2 inhibition showed dramatic resistance to fasting compared to control flies, while flies with blocked caspase activation had significantly shorter lifespans. Further studies can help understand the role of TOR signaling in mediating extrusions to achieve organ shrinkage in the adult *Drosophila* midgut.

699V Basal intercellular junctions integrate local cytoskeletal forces to regulate Hippo signalling in growing epithelia *Benjamin Kroeger*[1], Samuel Manning[1], Yoshana Fonseka[1], Kieran Harvey[1,2] 1) Monash Biomedical Discovery Institute, Monash University, Melbourne, Australia; 2) Peter MacCallum Cancer Centre, Melbourne, Australia

The Hippo signalling pathway is an evolutionarily conserved regulator of organ growth and homeostasis. By the assembly of pathway components in discrete subcellular domains, Hippo signalling can be either amplified or attenuated. The main subdomains previously associated with Hippo signalling in fly tissues are found at the apical portion of epithelial cells,

and include the adherens junctions and apical membranes. Hippo signalling can be modulated by cytoskeletal tension, which exerts forces at the adherens junctions and influences the subcellular localisation of the key Hippo pathway kinase, Warts.

Using a combination of molecular genetic approaches and high-resolution imaging, we discovered a novel subcellular domain where Warts is enriched: punctate, basal intercellular junctions. Using APEX2 transmission electron microscopy we found that in addition to its known enrichment at apical junctions, Warts accumulates in focal intercellular junctions at the basal-most region of epithelial tissues. Our genetic analyses show that the basal junctions are similar in composition to the belt-like adherens junctions. We reveal that Warts is dynamically recruited by LIM-family proteins to punctate basal junctions in response to local cytoskeletal forces, driven by myosin activity. By recruiting Warts basally, it is spatially restricted from activators of Hippo pathway signalling, which are enriched at the apical portion of epithelial cells. Our study sheds light on how the Hippo pathway integrates mechanical signals, and highlights previously overlooked aspects of cell biology and tissue dynamics that operate at the basal region of growing epithelial tissues.

700C Rab1 suggests a role for ER regulation in chromosomal separation during mitosis *Katie Rollins*, J. Todd Blankenship University of Denver

Mitotic spindles are often observed to be found in close association with the endoplasmic reticulum – however, the purpose of this association is less clear. The syncytial stage of fly embryogenesis is a period of rapid mitotic divisions, with the blastoderm stage marked by four rounds of divisions in a membrane-less syncytium near the cell cortex. These rapid divisions demonstrate major remodeling events of various membranous organelles, such as the nuclear envelope, the endoplasmic reticulum, and the deformation of the plasma membrane into cytokinetic-like furrows. Work from our lab, as well as others, has shown the contribution of multiple Rab-directed pathways to these processes, but relatively little work has examined how transformations in the ER contribute to successful mitoses. Here, we demonstrate that Rab1 controls ER to Golgi trafficking and is highly active within the Drosophila syncytium. Interestingly disrupting Rab1 results in severe mitotic failures, suggesting a role for the ER in permitting appropriate spindle function. In Rab1 knockdown embryos the endoplasmic reticulum accumulates in large spindle associated aggregates. This aggregation appears to be dynein-dependent, and disrupting the aggregation rescues mitotic function. We will also report on the mechanisms by which the over-accumulated ER affects the integrity of the spindle.

701A Understanding the role of Matrimony in suppressing the drive of the B chromosomes *Kaylah Samuelson*, Stacey Hanlon Department of Molecular and Cell Biology, University of Connecticut, Storrs, CT

The genome is constantly under attack by selfish genetic elements that bias their transmission to the next generation. A classic type of selfish element B chromosomes - supernumerary, nonessential chromosomes that can be detrimental to their host- were recently 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. Though it is clear that having only one functional copy of *mtrm* is necessary but not sufficient for this drive, the mechanism by which a reduction in Mtrm protein leads to drive of the B chromosomes is unknown. Mtrm has a critical function during female meiosis where it regulates Polo kinase (Polo) to promote centromeric cohesion of achiasmate (non-crossover) chromosomes and ensure their proper segregation during the meiotic divisions. This regulation requires Mtrm to bind to Polo through a highly conserved ST/P domain on Mtrm. One residue, T40, is particularly important for this interaction, as expression of a transgenic copy of Mtrm that has single amino acid change at that position (*mtrm*^{T40A}) is unable to rescue defects in the segregation of achiasmate chromosomes in a *mtrm*^{126} heterozygote. Since Mtrm's interaction with Polo is necessary to promote the proper segregation of achiasmate chromosomes, I hypothesize that this interaction may also be essential for Mtrm's ability to suppress the drive of the B chromosomes. To address this hypothesis, we will compare B chromosome transmission frequencies in a drive-competent (*mtrm*^{126}*/TM3, Sb Ser*) 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 determine if Mtrm's interaction with Polo is critical for the suppression of B chromosome meiotic drive, indicating that Mtrm's role in drive suppression and achiasmate chromosome segregation may be mechanistically similar. If the Mtrm-Polo interaction is not required for suppressing B chromosome meiotic drive, this result would lead us to test the necessity of other conserved Mtrm domains in its role as a drive suppressor. Overall, our work is uncovering the genetic mechanisms behind how the host genome protects itself against the proliferation of selfish genetic elements.

702B Crossover interference through ATR phosphorylation of Mei218 leading to phase separation of RING finger proteins Susan McMahan, Colette Anikwue, *Jeff Sekelsky* University of North Carolina at Chapel Hill

Sturtevant reported in his classic 1913 paper[1] that the presence of one meiotic crossover (CO) discourages others, a phenomenon Muller termed interference. More than 100 years later we still don't know the mechanism of interference. We propose a model in which induced phase separation of pro-CO proteins from the synaptonemal complex (SC), a

protein structure that forms between paired homologous chromosomes, followed by coarsening, these pro-CO proteins to accumulate at a single site or widely separated sites.

In *Drosophila*, ~20-24 double-strand breaks (DSBs) are made per meiosis, distributed uniformly throughout the euchromatin (at large scale). About 1/4 of these are designated to become COs and the rest are repaired as non-crossovers. In her EM studies of recombination *Drosophila*, Carpenter[2] discovered a CO-associated structure she called the recombination nodule (RN), a 100 nM sphere that sits on top of the SC. The proposal by Dernburg and colleagues that the SC had liquid-like properties[3] and that recombination factors can move within this compartment[4] suggest the RN may be a droplet containing CO-designating proteins.

We propose that the pro-CO mei-MCM complex (Mei-217, Mei-218, and Rec)[5] is localized to DSB sites. A 2nd set of pro-CO factors, the RING-finger proteins Vilya, Narya, and Nenya[6,7] (here abbreviated VNN), move within the liquid SC. Phosphorylation of the intrinsically disordered domain of Mei-218 by Mei-41 (ATR kinase) promotes physical interaction with VNN. VNN accumulates and dewets from the SC. Exchange between nearby occurs through the SC, but larger accumulations become more stable, so that each SC (*i.e.*, each chromosome arm) eventually has a single VNN or widely separated VNN foci. These sites are designated as COs. This process naturally leads to crossover assurance - the guarantee that each pair of homologous chromosomes (excepting the *4* in *Drosophila melanogaster*) almost always get at least one crossover to promote accurate segregation. In our view, interference is merely a consequence of the assurance process.

We made numerous *in situ* mutations in *mei-218* to alter ATR phosphorylation sites. Mutation of S/T to the phosphomimetic residue D results in decreased COs and loss of interference, with increasing severity for 3, 5, or 8 sites mutated. In the most severe case there is high negative interference, where double-COs in two adjacent intervals are significantly more frequent than expected if the intervals are independent of one another. In addition to these data, progress toward dissection of different phosphorylation sites and mapping of interactions between VNN proteins and phospho-Mei-218 will be presented.

[1] Sturtevant 1913 *J Exp Biol.*; [2] Carpenter 1979 *Chromosoma*; [3] Rog *et al.* 2017 *Elife*; [4] Zhang *et al.* 2018 *Elife.* [5] Kohl *et al.* 2012 *Science*; [6] Lake *et al.* 2015 *eLife*; [7] Lake *et al.* 2019 *PLOS Gen*

703C Meiotic Crossover Patterning: The Centromere Effect *Nila Pazhayam*, Jeff Sekelsky University of North Carolina at Chapel Hill

Crossing-over between homologous chromosomes is a critical part of meiosis that prevents aneuploidy by promoting proper segregation of chromosomes. 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 this patterning event are poorly understood. To address this gap in knowledge, I will investigate 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 will study differences in the number and positioning of DSBs in flies with heterochromatin defects, as compared to wild type. Recent work from our lab has also suggested that the CE in beta heterochromatin and proximal euchromatin is distance dependent. I will investigate whether this distance effect is based on distance from the centromere, or alpha heterochromatin, by deleting a large satellite array from the pericentric regions of the *X* chromosome. This will not only remove a large segment of alpha heterochromatin, but also move beta heterochromatin and proximal euchromatin closer to the centromere. Measuring CO rates in these regions will determine how the CE in proximal euchromatin and beta heterochromatin is influenced by proximity to alpha heterochromatin and the centromere. Another avenue that I plan on investigating is the sensitivity of the CE to the number of centromeres. I will study this by measuring crossover rates in flies that have a reduced total number of centromeres through the use of compound chromosomes. Through these and other methods, my overarching goal is to gain more understanding of the mechanisms at work behind crossover patterning, particularly the exclusion of crossovers near the centromere.

704A Mechanisms and regulation of meiotic recombination: a whole-genome approach *Carolyn Turcotte*, Jeff Sekelsky University of North Carolina - Chapel Hill

Meiosis is a specialized form of cell division that generates haploid gametes from diploid cells. Crossovers between homologous chromosomes are essential to accurate chromosome segregation during meiosis, and chromosome missegregation leads to miscarriage and chromosomal disorders such as Down syndrome. Crossovers are generated via homologous recombination, a process that repairs double-strand DNA breaks (DSBs) using a homologous template. Many more breaks than crossovers are formed, and the number and position of crossovers are highly regulated in a phenomenon termed "crossover patterning." Despite crossover patterning's discovery over a century ago, the mechanisms that govern it remain unclear. A classical model for homologous recombination suggests that all crossovers are derived from a single symmetrical intermediate, with noncrossover products being derived from an earlier intermediate. The multiple pathways within homologous recombination can be traced via heteroduplex DNA (hDNA), DNA in which the strands are derived from different parent molecules (e.g. homologous chromosomes). Normally, SNPs and indels between the strands in hDNA are repaired by mismatch repair to form gene conversions or restorations of the original sequence. Our lab has found that *Drosophila Xpc ; Msh6* mutants, deficient in both canonical and short-patch mismatch repair, exhibit continuous hDNA tracts, making Drosophila the only metazoan thus far in which this has been achievable. Our lab has studied these hDNA tracts at a test locus and found hDNA signatures conflicting with the classical recombination model. However, much more extensive analysis than is possible at this test locus is required to revise this model. I am conducting whole-genome sequencing of hDNA to determine if the signatures seen at a single locus are consistent genome-wide, and whether certain hDNA signatures are localized to specific regions of the genome. This analysis will shed light on meiotic recombination mechanism and will inform if and how pathway choices in recombination differ in different genomic contexts. Understanding meiotic recombination mechanism and pathway choice is crucial to determining its connection to crossover patterning and proper homolog segregation.

705B Tissue specific requirements of the Rcd4:Ana3 sub-complex in *Drosophila* centriole assembly *Pallavi Panda*[1], Levente Kovacs [1], Nikola Dzhindzhe[2], Agnieszka Fatalska[2], Veronica Persico[3], Maria Giovanna Riparbelli[3], Giuliano Callaini[3], David M Glover[1] 1) California Institute of Technology, United States; 2) University of Cambridge, United Kingdom; 3) University of Siena, Italy

Centrosomes are macromolecular protein complexes assembled through precise protein-protein interactions. Such proteins regulating centriole duplication appear to assemble and function in sub-complexes; Sas6 bound to phosphorylated Ana2 initiates pro-centriole formation; Sas4 interacts with centriolar microtubules; the Cep135:Ana1:Asl network promotes centriole to centrosome conversion enabling recruitment of Plk4 and peri-centriolar material. We have recently described a new sub-complex formed in the Drosophila centriole between Rcd4 and the C-terminal half of Ana3. As a poorly characterized Drosophila centriolar protein, we describe null and hypomorphic rcd4 mutant flies that exhibit fewer centrioles and aberrant mitoses in several somatic tissues and show structural defects in the basal bodies of sensory organs. Structured illumination microscopy of cultured cell centrioles reveals that Rcd4 and Ana3 load onto zone I of the centriole during interphase, where Ana3 recruitment precedes Rcd4. We find that neither Ana3 nor Rcd4 participates directly in the mitotic conversion of centrioles to centrosomes, but assembly of the Rcd4:Ana3 sub-complex is a pre-requirement for the recruitment of Ana1 and subsequently for the transition to the final stage of centriole to centrosome conversion. In contrast, we show differing requirements of the sub-complex for centriole development in the male germline. Whereas ana3 mutants are male sterile indicating that Ana3 is essential for centriole formation during spermatogenesis, rcd4 mutants are male fertile, yet display a multitude of centriolar defects. Contrary to our findings in somatic cells, elongated centrioles of ana3 and rcd4 mutant spermatocytes still accumulate Ana1 and Asl. Moreover, it appears that while Rcd4 is not required for the initial recruitment of Ana3 to spermatogonia centrioles, it is needed for the recruitment/maintenance and accurate localization of Ana3 upon centriole elongation in primary spermatocytes. Taken together, our results suggest tissue-specific roles of Rcd4 and Ana3 in centriole and basal body formation.

706C Functional domains of the Ana1 centriole protein and their regulation by mitotic protein kinases and phosphatases *Agota Nagy*[1], Nikola Dzhindzhev[2], Pallavi Panda[1], Levente Kovacs[1], Helene Rangone[2], Jingyan Fu[2,3], Zoltan Lipinszki[2,4], David Glover[1,2] 1) Division of Biology and Biological Engineering, California Institute of Technology, Pasadena, California, USA; 2) Department of Genetics, University of Cambridge, Cambridge, UK; 3) State Key Laboratory of Agrobiotechnology, College of Biological Sciences, China Agricultural University, Beijing, China; 4) Biological Research Centre, Institute of Biochemistry, MTA Lendület Laboratory of Cell Cycle Regulation, Szeged, Hungary

Centrioles are evolutionary conserved, 9-fold symmetrical structures that form the core component of centrosomes and also serve as basal bodies for cilia and flagella. They play critical roles in cell division, motility and signalling. The assembly of centrioles through the cell cycle is tightly regulated, leading to their elongation in the G2 phase followed by their conversion to centrosomes during mitosis. These processes require the progressive assembly of a network of interacting proteins, which extend from the inside to the outside of the centriole. Here we focus on Ana1, the protein that links the inner and outer centriole components of this molecular network. We wish to understand the independent function of Ana1's domains and to this end, have created transgenic flies expressing N-and C-terminal fragments of different lengths. We separately express these fragments in different *ana1* mutant backgrounds and determine the extent to which they can function independently to drive centriole duplication and elongation in different tissues. We are

determining how the interactions of Ana1's different functional domains with other key centriole proteins are dependent upon phosphorylation by mitotic protein kinases for completion of the procentriole, its disengagement from the mother centriole, and recruitment of peri-centriolar material and how protein phosphatases ensure the cyclical nature of some of these interactions.

707A Cohesin dynamics during meiotic prophase in Drosophila oocytes *Muhammad Abdul Haseeb*, Sharon Bickel Dartmouth College

Premature loss of sister chromatid cohesion during the meiotic arrest in human oocytes is a major contributor to the maternal age effect, a phenomenon in which the probability of an aneuploid pregnancy increases as a woman ages. Our laboratory has previously uncovered evidence for a cohesion rejuvenation program in Drosophila oocytes that operates during prophase, after cohesive linkages are originally established during S phase. To better understand the behavior of rejuvenation-specific cohesin, we designed an "allele-switch" transgene that expresses endogenous levels of the cohesin subunit Smc1 tagged with mCherry which is flanked by FRT sites. Induction of Flippase using the prophase specific matα-Gal4 driver causes excision of the mCherry tag, resulting in expression of Smc1 tagged with superfolder GFP (sGFP). This tool allows us to specifically visualize chromatin-associated cohesin that is synthesized after the canonical cohesion establishment that occurs during oocyte DNA replication. Upon switch induction with the matα driver, newly synthesized Smc1-sGFP begins to load onto the meiotic chromosomes as early as oogenesis stage 2. Meiotic prophase lasts ~6 days in Drosophila oocytes. Surprisingly, almost the entire cohesin population appears to turn over in < 2 days (by oogenesis stage 5). Turnover at centromeres is slower than at arms. Matα-induced knockdown (KD) of the cohesin subunit Smc3 abolishes chromosomal loading of newly synthesized Smc1-sGFP. This failure to load rejuvenation-specific cohesin during prophase leads to premature loss of arm cohesion in metaphase I arrested oocytes, as evidenced by fluorescence in situ hybridization that uses Oligo-PAINT probes to visualize X-chromosome arm cohesion. Together these results indicate that rejuvenation in Drosophila oocytes is required to maintain levels of cohesion that are sufficient for accurate chromosome segregation. Future experiments will utilize the allele-switch transgene to investigate cohesin loading dynamics when cohesion regulators or rejuvenation specific proteins are knocked down during prophase. These experiments will allow us to better understand the mechanism(s) underlying cohesion rejuvenation during meiotic prophase in Drosophila oocytes.

708B Discs large licenses Pins to orient mitotic spindles *Kathryn Neville*, Dan Bergstralh University of Rochester

Oriented cell divisions are critical to animal development. Division orientation determines the placement of daughter cells, and thereby promotes cell diversity and helps to organize and expand tissues. The direction in which a cell divides is established by the orientation of the mitotic spindle at metaphase. Spindle orientation in animals typically relies on an evolutionarily conserved machine comprised of at least four proteins: in flies, these are called Partner of Inscuteable (Pins), Gαi, Mushroom body defective (Mud), and Dynein. The canonical model is that Pins acts as a cortical anchor for Mud and Dynein, which exert a pulling force on astral microtubules that reels the spindle into alignment. Another established, but poorly understood, component of this machinery is Discs large (Dlg), an apical-basal polarity factor that interacts with phosphorylated Pins. Whereas previous work from our lab and others suggestes that Dlg is required for Pins localization, I show here that Pins localization is unaffected by the loss of Dlg. My results indicate that Dlg works to activate, rather than localize Pins function. These results suggest that the canonical model is incomplete, raising the question of how the spindle orientation machinery is regulated.

709C Evolutionarily conserved midbody reorganization precedes ring canal formation during gametogenesis *Kari Price*, Dyuthi Tharakan, Lynn Cooley Yale School of Medicine

During gametogenesis, male and female germ cells develop as syncytia connected by intercellular bridges that facilitate cell-cell communication and the transport of molecules and organelles between sister cells. Functional studies demonstrate the requirement of germline bridges for fertility, yet the mechanism underlying their formation is still unclear. Immunofluorescence studies of fixed Drosophila tissues have suggested that these bridges, otherwise known as ring canals, arise from arrested constriction of the contractile ring. In contrast, studies in the mouse testis have suggested that germline bridges are derived from the midbody matrix. These observations, combined with the compositional differences between Drosophila male and female ring canals and mouse and Drosophila male ring canals, raise the question of whether there is one underlying, conserved mechanism of ring canal formation. To investigate how ring canals are formed, we performed time-lapse imaging of the conserved ring canal component Pavarotti/MKLP1/kinesin-6 during the incomplete mitotic divisions of the male and female Drosophila germlines. In both testes and ovaries, rather than contractile ring arrest, contractile rings constrict completely to form a dense, transient midbody intermediate that reorganizes into an open ring canal in less than one hour. The midbody-to-ring canal transition appears to be a conserved feature of gametogenesis as we also observed kinesin-6-labeled midbodies in fixed preparations of mouse and *Hydra vulgaris* male germlines.

To further characterize the midbody-to-ring canal transition, we imaged known midbody ring components in the

Drosophila testis. We observed that the germline midbody is compositionally similar to, but functionally distinct from, the midbodies formed during complete cytokinesis. Known midbody ring components Septin 2 and Citron kinase/Sticky localize to the germline midbody, but as expected, germline midbodies fail to initiate microtubule severing and abscission. Interestingly, in addition to the midbody ring localization, Sticky localizes to a subset of ring canals, presumably nascent ring canals, but not mature ring canals suggesting a possible role in the midbody-to-ring canal transition. In fact, knockdown of *sticky* in the male germline (*nos>sticky RNAi)* results in persistent midbody-like foci. We are now working to understand the precise role of Sticky during the midbody-to-ring canal transition and identify additional components required.

710A Functional Analysis of Bloom Syndrome Helicase in Development and DNA Repair *Colleen Bereda*, Evan Dewey, Jeff Sekelsky University of North Carolina at Chapel Hill, Chapel Hill, NC

Bloom Syndrome is a rare autosomal recessive disorder in humans caused by mutation of the *BLM* gene that leads to increased genome instability and cancer. The *BLM* gene codes for a helicase (BLM) that works together with Topoisomerase 3-alpha (Top3α) in homology-directed repair of DNA. Top3α assists by directly binding to BLM and helping to release the torsional stress on DNA as BLM helicase unwinds recombination intermediates. These proteins preserve genome stability and have been shown in many organisms to operate together in the prevention of detrimental mitotic (non-meiotic) crossovers via two main DNA repair pathways, Synthesis-Dependent Strand Annealing and double-Holliday Junction dissolution. In *Drosophila*, BLM (known as Blm) also has roles in proper meiotic chromosome segregation and rapid cell cycle progression of the developing embryo. To investigate the Blm-Top3α interaction in DNA repair, I performed a yeast 2-hybrid (Y2H) assay using the *Drosophila* proteins. I found interaction was specific to certain regions of Blm, with the strongest observed for a C-terminal region conserved among several *Drosophila* species, amino acids (aa) 1381-1487. To further decipher Blm roles, I created specific *Blm* deletions of regions from my Y2H data via CRISPR/Cas9 editing and tested various Blm roles *in vivo*. First I examined the role of Blm in promoting embryonic development. While deletion of aa 1-240 did not have an effect, deletion of aa 576-720 had substantial impact with most embryos failing to hatch. I then assessed the effects of Blm deletions on known Blm roles in meiotic chromosome segregation via a nondisjunction assay. Both aa 1-240 and aa 576-720 deletions were not significantly different from our wild-type control in contrast to my embryonic development data. This result suggests deletion of aa 576-720 is a separation-of-function mutation. Future studies will assess importance of these Blm regions in preventing mitotic crossovers and determining their importance to Blm function in DNA repair. I will also further explore Blm aa 576-720 and the relevance of predicted ATR/ATM (*Drosophila* Mei-41 and Tefu; regulators of DNA repair) phosphorylation sites within this region for proper Blm function in embryonic development, meiotic segregation, and DNA repair. By characterizing the functions of Blm in *Drosophila*, we will better understand *BLM* function within humans and the detrimental health effects of *BLM* mutations.

711B Alternative End Joining Preferences in RPA-Deficient Drosophila *Jacob Zuckerman*, Terrence Hanscom, Nick Woodward, Vicki Do, Mitch McVey Department of Biology, Tufts University, Medford, MA

DNA double-strand breaks can be repaired using several different repair pathways. These include homologous recombination, classical-non homologous end joining, and a third, less well-understood set of mechanisms known as alternative end-joining. Alternative end joining repair is extremely inaccurate, causing insertions, deletions, and/or indels and has been linked to several different types of cancers. One type of alternative end joining, known as synthesis-dependent microhomology mediated end-joining (SD-MMEJ), occurs when short repeated sequences near the break site form secondary structures that prime nascent synthesis of microhomologies that are then used to repair the DNA. In yeast, the replication protein A (RPA) heterotrimer has been shown to prevent another similar alternative end-joining pathway, microhomology mediated end-joining. To bind efficiently, RPA requires approximately 30 base pairs of single stranded DNA. Previous data from our lab demonstrate that movement of the short-repeated sequences to a distance greater than 30 bp from the break site reduces the frequency of SD-MMEJ usage from 15% to <1%. Therefore, we hypothesize that RPA that may inhibit SD-MMEJ by binding to short stretches of single stranded DNA and preventing secondary structures from forming. To test this, we have obtained a fly stock with a *P* element inserted in the 5' UTR of the *RPA70* gene, which severely decreases RPA70 expression. Cas9-induced breaks will be generated in a set of reporter plasmids that are injected into embryos from this stock. Deep sequencing and computational analysis of the inaccurate repair products will allow us to discern the role of RPA in SD-MMEJ repair. In addition, these studies may shed light on alternative end joining repair processes in cancer cells, which likely experience RPA exhaustion due to the accumulation of single-stranded DNA during perturbed DNA replication.

712C Defining Mitotic Crossover Mechanisms Using CRISPR/Cas9 and Bloom Syndrome Helicase *Evan Dewey*, Jeff Sekelsky University of North Carolina-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 "scars" in the form 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 to integrate desired mutations or corrections in genome editing, but the precise CRISPR/Cas9 HDR mechanisms remain elusive. I have begun to test mitotic crossover mechanisms using both CRISPR/Cas9 induced double strand breaks and mutation of Bloom Syndrome Helicase (*Blm*), a key HDR regulator that prevents mitotic crossovers. Through combination with total mismatch repair (MMR) knockout only possible in *Drosophila* (through both *Msh6* canonical and backup *Xpc* short-patch pathways), it is now possible to analyze resulting mitotic crossover products using Sanger sequencing for CRISPR/Cas9 induced double strand breaks and whole genome sequencing for *Blm* mutants. Using these tools, I have begun to accurately define mitotic crossover mechanisms for the first time in a multicellular organism, with an unligated double Holliday junction resolution model indicated as the primary mechanism. Unexpectedly, combination of *Blm* and MMR knockout also appears to substantially increase the rate of mitotic crossovers compared to knockout of either alone, indicating a previously uncharacterized genetic interaction between *Blm* and MMR components in HDR and prevention of mitotic crossovers. This work has begun to enhance understanding of how DNA is repaired in both CRISPR and *Blm* mutant contexts, expanding knowledge of how mitotic crossovers lead to genome instability, identifying previously uncharacterized interactions in HDR and mitotic crossover regulation, and providing better understanding of how to beneficially utilize mitotic crossover mechanisms in genome editing.

713A The Krüppel-like factor Cabut has cell cycle regulatory properties similar to E2F1 *Peng Zhang*[1,2], Alexia J. Katzaroff[3,4], Laura A. Buttitta[4], Yiqin Ma[1,2], Huaqi Jiang[4], Derek W. Nickerson[4], Jan Inge Øvrebø[1,2], Bruce A. Edgar[1,2,3,4] 1) Huntsman Cancer Institute, University of Utah, Salt Lake City, UT 84112; 2) Department of Oncological Sciences, University of Utah, Salt Lake City, UT 84112; 3) Molecular and Cellular Biology Program, University of Washington, Seattle, WA 98195; 4) Division of Basic Sciences, Fred Hutchinson Cancer Research Center, Seattle, WA 98109

Using a gain-of-function screen in *Drosophila*, we identified the Krüppel-like factor Cabut (Cbt) as a positive regulator of cell cycle gene expression and cell proliferation. Enforced cbt expression is sufficient to induce an extra cell division in the differentiating fly wing or eye, and also promotes intestinal stem cell divisions in the adult gut. Although inappropriate cell proliferation also results from forced expression of the E2f1 transcription factor or its target, Cyclin E, Cbt does not increase E2F1 or Cyclin E activity. Instead, Cbt regulates a large set of E2F1 target genes independently of E2F1, and our data suggest that Cbt acts via distinct binding sites in target gene promoters. Although Cbt was not required for cell proliferation during wing or eye development, Cbt is required for normal intestinal stem cell divisions in the midgut, which expresses E2F1 at relatively low levels. The E2F1-like functions of Cbt identify a distinct mechanism for cell cycle regulation that may be important in certain normal cell cycles, or in cells that cycle inappropriately, such as cancer cells. That Cabut has E2F-like activity is especially interesting when one considers that the E2Fs, which are the best characterized transcriptional regulators of the eukaryotic cell cycle, are not actually essential for cell cycle gene transcription or cell cycle progression. Hence, it is clear that other transcription factors must also regulate the transcription of the 300 to 400 genes necessary for DNA replication and mitosis. Cabut appears to be one of these missing transcription factors that can complement E2F1.

714B Excess histone H3 is a Chk1 inhibitor that controls embryonic cell cycle progression Yuki Shinso, *Amanda Amodeo* Dartmouth College

The early embryos of many species including Drosophila spend the first few hours of development undergoing a series of rapid cell cycles to generate the large number of cells required for embryogenesis. These early reductive divisions are largely transcriptionally silenced and therefore depend upon pools of maternally provided components. Cessation of the rapid cell cycle program at the mid-blastula transition (MBT) is dependent on achieving a sufficient nuclear to cytoplasmic (N/C) ratio and the activity of the DNA-damage checkpoint kinase, Chk1 (grapes). How Chk1 activity is coupled to the N/C ratio has remained unclear. We have shown that large stores of extra-nucleosomal histone H3 accumulate in the early nuclei because the maternally provided histone pools vastly exceed the amount of DNA in the early embryo. As the N/C ratio increases these pools are exhausted. At the same time, histone H3 is a known Chk1 substrate. Therefore, we hypothesized that in the early nuclei H3 may compete with Chk1's cell cycle regulatory targets (CDC25-string and twine) to prevent cell cycle slowing before the correct N/C ratio is reached at the MBT. To test this we expressed a version of the H3 N-terminal tail that contains the Chk1 binding site, but cannot be incorporated into chromatin in the pre-MBT embryo. We found that this 47-amino acid fragment is sufficient to delay cell cycle slowing and reduce Chk1 activity in vitro and in vivo. Mutation of the Chk1 phosphosite in the context of the full length H3 protein is sufficient to lengthen the cell cycle and accelerate the onset of the MBT. Together our data support a model in which hyper-abundant histone H3 in the early embryo competitively inhibits Chk1 to prevent cell cycle slowing until a sufficient N/C ratio is achieved. We are currently working to determine if H3 inhibits Chk1 in other developmental contexts including in response to DNA-damage.

715C Regulation of induced endocycling cells and their effects on tissue growth *Hunter Herriage*, Yi-Ting Huang, Savanna Brown, Brian Calvi Indiana University

Non-canonical variations of the cell cycle are important in development and disease. One of these variations is the endocycle, which results in large, polyploid cells that arise through inhibition of mitosis. In addition to these developmental endocycling cells (devECs), conditional signals can result in induced endocycling cells (iECs), such as during wound healing, aging, or cancer. We have previously shown in *Drosophila* that devECs and iECs repress the apoptotic response to DNA damage. With the Walczak lab, we have also shown that transient iECs in both flies and humans can undergo error-prone mitosis (RTM). These data are consistent with mounting evidence that cancer cells can transiently enter the endocycle in response to genotoxic therapies, and can then switch back to error-prone, polyploid mitoses that contribute to genome instability and tumor regrowth. Much remains unknown, however, about how the growth of transient iECs is regulated and how iECs affect tissue homeostasis and tumorigenesis.
We will report our progress using *Drosophila* as an *in vivo* model system to decipher how endocycles contribute to normal and abnormal tissue growth. Particularly, we will discuss our investigations into the possible synergies between pro-growth mutations and different variant cell cycles. Although iECs repress apoptosis, we will present evidence that a subset may senesce, depending on developmental context. This work will lead to a greater understanding of growth, proliferation, and checkpoint regulation in polyploid cells, and their effects on tissue homeostasis and cancer.

716A Defining the Dynamics of Transcriptional Bursting in Developing *Drosophila* legs *Rina Helt*, Elizabeth Urban, Robert Johnston 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 90-120 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.

717B Molecular genetic analysis of the mutation *l.3.2* by undergraduates participating in a *Drosophila* CURE Cory Evans[1], *Veronica Casarez*[1], Kayla Bieser[2], Jacob Kagey[3] 1) Loyola Marymount University; 2) Nevada State College; 3) University of Detroit Mercy

The mutation *l.3.2* was identified in an FLP/FRT-based genetic screen for novel regulators of cell growth located on the right arm of chromosome 2 (2R). Because abnormal growth phenotypes associated with new mutations may be suppressed by the parallel activation of apoptosis, EMS was used to mutagenize an FRT42D chromosome already carrying the *Dark*^{82} mutation, which strongly suppresses apoptosis. This screen successfully identified several new mutations affecting cell growth including *l.3.2*. Some of these mutations have been or are being characterized by undergraduate students participating in FlyCURE, a course-based undergraduate research experience (CURE) that is being implemented at several partnering colleges and universities across the country. Here we describe the FlyCURE analysis of mutation *l.3.2*., which behaves zygotically as a recessive lethal. *l.3.2* is likely also a cell-lethal mutation, as flies with mitotic mutant clones created in the developing eye (using *ey-FLP*) are pupal lethal and exhibit an almost complete lack of developing head structures. Complementation mapping of the *l.3.2* recessive-lethal phenotype using the Bloomington *Drosophila* Stock Center 2R Deficiency Kit identified two non-complementing deficiencies. These deficiencies overlap in the cytological interval 50A7-50A13, a small chromosomal region containing thirteen genes. A lethal allele of one of these genes, *centromere identifier* (*cid*), failed to complement *l.3.2*, suggesting that *l.3.2* is an allele of *cid*. DNA sequence analysis of the *cid* locus in the *l.3.2* genetic background is underway to confirm the identity of the *l.3.2* gene and determine the molecular nature of the mutation.

718C Probing the Temporal Regulation of Hatching in *D. melanogaster* *Alexandra (Olenka) Jain*[1,2], Stanislav Shvartsman [1,2] 1) Princeton University; 2) Lewis-Sigler Institute of Integrative Genomics

The ability to regulate the timing of irreversible transitions between sequential developmental states is one of the

most critical features of any developmental process. Understanding what controls the variation in the duration of a developmental stage is an important question in developmental biology. We address this question using *Drosophila melanogaster* embryogenesis because it has an extremely well-defined start and end. We describe a straightforward approach to characterize the inter-embryonic variability in the duration of embryogenesis by measuring the time of hatching events in cohorts of synchronized embryos. This approach is based on videos taken from oviposition until hatching, automated detection of hatching events, and nonparametric statistical analysis of the hatching time distributions. We demonstrate how the empirical distribution functions of hatching times can be used to study the effects of maternal age and genotype on the duration of embryogenesis. Specifically, this approach can be used to characterize the hatching distribution function of flies with a mutation in the *amontillado* gene, which has been shown to be important for the duration of embryogenesis (Siekhaus & Fuller, 1999). In addition to this, we revisit the connection between hatching and molecular and motor activities in the embryo.

719A Identification of Apoptosis and Junctional Tension as Pro-tumoral Factors in Drosophila *Marianne Montemurro*, Bruno Monier, Magali Suzanne Centre de Biologie Integrative CBI - Molecular, Cellular and Developmental Biology Unit - UMR 5077

Cancer is a largely widespread pathology that corresponds to an overproliferation of cells that could finally invade others tissues. Tumors develop through three increasingly aggressive steps: (1) hyperplasia, which corresponds to cells overproliferation without any modification of epithelial properties; (2) neoplasia, during which cells can acquire a more mesenchymal phenotype, and finally (3) metastasis, when cells leave the primary tumor, migrate and form secondary tumors.
Tumor development can be influenced by mutations (or combination of mutations) but also by external factors, such as extracellular matrix rigidity. However, a comprehensive understanding of the factors driving tumor evolution is still lacking. **My project aims to use *Drosophila* to identify unexpected factors that could influence tumor development, and more specifically the hyperplasia/neoplasia transition, a critical step in tumor aggressiveness.** After an in-depth characterization of tumor progression at cellular level at successive timepoints, I selected two complementary genetic contexts for further analysis: a strictly hyperplasic tumor ("*Yorkie* overexpression" context) and an initially hyperplasic tumor that eventually evolves in neoplasia ("*Avalanche* loss-of-function" context).
Strikingly, I identified two new and unexpected factors involved in tumor progression and aggressiveness: apoptosis and tissue tension. Indeed, while tumor aggressiveness coincides with a high level of apoptotic cells, abolishing apoptosis in *Avalanche* tumors strongly decreases the hyperplasia/neoplasia shift, while introducing cell death in *Yorkie* tumors converts hyperplastic tissues into neoplastic ones. Hence, in those two different contexts, modulation of apoptosis surprisingly favors the hyperplasia/neoplasia transition. Moreover, I found that tumor aggressiveness is associated with high tissue tension and that increasing junctional tension (by modulating a Myosin II regulator) is sufficient to induce a hyperplasia/neoplasia transition in a tumor context normally strictly hyperplasic (Yorkie's context). I am now characterizing whether cell death and tissue mechanics are linked or whether they act independently of one another to drive tumor progression.

720B Examining the synthetic lethality between BRCA2 and methyl and ethyl Paraben *Zainab Rizik*, Nicole Banuelos, Blake Riggs, Lela Legesse San Francisco State University

There have been increases in incidences of breast cancer since 1970 due to increased exposure to chemicals in our daily lives. Parabens are found in our everyday lives and are disproportionately pushed on poor communities via personal products and food. Recent studies have found that parabens mimic estrogen and attach to the receptors of breast cancer cells and cause them to proliferate, however the pathway and additional targets are poorly understood. The BRCA2 gene plays a vital role in providing instructions for creating a tumor suppressor protein preventing cells from excessively multiplying. When there is a mutation in this gene, it could cause breast cancer due to the loss of function of the protein. This experiment analyzes the effect of the toxins methyl and ethyl paraben on organisms with BRCA2 gene mutations using *Drosophila melanogaster*. To study this, the survivability of homozygous BRCA2 mutant, we exposed larvae to different concentrations of parabens (300, 700, 1000 mg/l) and determined the survival rate. Overall, it was found that there was increased synthetic lethality in flies and at increased doses of parabens. These findings will pave the way in lowering the amount of future breast cancer cases caused by the use of methyl and ethyl paraben.

721C Candidate ion channel screen identifies modifiers of brain tumor size *Isabella Maag*, Ashley Bielawski, Hannah Christman, Beverly Piggott University of Montana, Missoula, MT

Ion channel function is critical for normal development and prevention of disease. These functions include maintaining membrane potential, controlling the flow of ions across the membrane, and regulating cell volume. Ion channels have well described roles in excitable cells where they are essential for establishing membrane potentials, the firing of action potentials and contraction of muscles among other functions, but their role in non-excitable cells is less defined. While ion channels are found in neural progenitors and upregulated in cancer, their roles, in these tissues, are not well understood. Previous work has found that reduction of voltage-gated sodium channel Para reduces brain tumor size. To

further define the bioelectric landscape of proliferation, we sought to identify ion channels that influence tumor size and potentially proliferation. Using a Deadpan overexpression (Dpn^{OE}) brain tumor model which displays hyper-proliferation, we conducted a RNAi candidate ion channel screen to identify modifiers of brain volume. This screen has revealed a number of ion channels whose knockdown, significantly alters brain volume. This suggests that these ion channels may regulate important aspects of tumorigenesis. As ion channels are druggable targets, identifying modifiers of tumor size may represent new therapeutic sites of intervention.

722A Yorkie dependent transcriptional network promotes tumor growth *arushi rai*[1], Indrayani Waghmare[3], Amit Singh[1,2], Madhuri Kango-Singh[1,2] 1) Department of Biology, University of Dayton, Dayton, OH; 2) Integrative Science and Engineering Center, University of Dayton, Dayton, OH; 3) Department of Cell and Developmental Biology Vanderbilt University, Nashville, TN

Studies in *Drosophila* and other tumor models have revealed cancer promoting signaling interactions and transcriptional addictions in tumors cells. The Hippo pathway effector, Yorkie (Yki) is a key mediator of such interactions, and presents an attractive opportunity to study transcriptional dependencies in cancer cells. The Ras^{V12}, $scrib^{-/-}$ tumor mosaic model is well-established and shows activation of oncogenic Ras in the background of impaired apical-basal polarity. This model is widely used study molecular mechanisms and signaling events downstream of the oncogenic Ras and Ras-mediated Yorkie (Yki) activation in Ras^{V12}, $scrib^{-/-}$ tumor cells. Previously, we have shown that in Ras^{V12}, $scrib^{-/-}$ cells Wingless (Wg), Caspases (e.g., the initiator caspase Dronc) and JNK are activated to promote tumorigenesis through their non-apoptotic roles. Amongst these, Wg/Wnt pathway is known to act via canonical and non-canonical pathways during development and cancer, and interact with Yki to promote cancer growth. Genetic epistasis showed that Wg acts upstream of Caspases, JNK and Yki, and downregulation of Wg reduced tumor growth by downregulation of Caspases, JNK and Yki reporters. Our goal is to further understand how the two evolutionarily conserved signaling pathways i.e., Hippo and Wingless crosstalk and interact with each other to regulate tumor growth. To understand this intricate wiring of Wingless-Yorkie during tumor growth and invasion, we will use the Ras^{V12}, $scrib^{-/-}$ tumor model in *Drosophila* imaginal discs. Preliminary data showed that *wg* transcriptional reporters are upregulated in Ras^{V12}, $scrib^{-/-}$ cells, suggesting that increased accumulation of Wg may be due to increased transcription. In other contexts, *wg* is shown as a transcriptional target of Yki. Therefore, we will test for (a) the effects of Yorkie protein, the main effector molecule of Hippo pathway, on *wg* transcription and expression of other Wg pathway components by reporter assays, and qRT-PCR-based approaches, and (b) 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.

723B Fmi-mediated cell polarity and adhesion are critical during cell competition and tumorigenesis in *Drosophila* *Pablo Sanchez Bosch*, Bomsoo Cho, Jeffrey Axelrod Stanford University

Cell competition is a process by which cells with a competitive advantage (winners) eliminate less fit neighboring cells (losers). This phenomenon was first seen when cells harboring ribosomal protein mutations were eliminated by their WT neighbors. Since that first observation in 1975, cell competition has been linked to mutations in many other genes, i.e. tumor suppressors, oncogenes, or cell polarity components. Even though the outcome is always the elimination of the less fit cells, very little is still known about the mechanisms leading to the selection of fitter cells and elimination of the losers. Tumors have been seen taking advantage of cell competition to outcompete the surrounding WT tissue and promote survival and local invasion. Therefore, understanding the molecular cues of cell competition will give us not only insight into a key developmental process but a deeper understanding of tumorigenesis.

We have found that Fmi, a key component of the planar cell polarity (PCP) pathway, plays a critical role in various cell competition scenarios and Ras^{V12}-dependent tumorigenesis. Intercellular Fmi homodimers scaffold the assembly of the other core PCP proteins and mediate asymmetric signaling between the core PCP proteins. PCP signaling polarizes cells within the plane of the epithelium to orient cellular structures, cell divisions, and cell movement during development and homeostasis. In addition to establishing planar polarity, numerous reports have linked PCP signaling to cancer.

Our experiments show that Fmi is critical for winner cells in cell competition and tumorigenesis. Tumors and winner cells lacking Fmi lose their ability to eliminate neighboring loser cells. Moreover, *fmi*-null winners undergo apoptosis. Removing Fmi from loser cells does not have any effect on cell competition outcomes, suggesting that Fmi is only critical by winners to outcompete losers. Our findings suggest that Fmi acts as a scaffolding and signaling hub, where Fmi molecules, via PCP or other signaling networks, allow for efficient cell communication, necessary for the elimination of loser cells.

Our results are striking in that Fmi is critical in all the study cases we've tackled, and establish the necessary background for comprehending the mechanisms underlying cell-cell communication during both developmental cell competition and tumorigenesis.

724C Modulation of Hippo signaling by Mnat9 N-acetyltransferase for normal growth and tumorigenesis in *Drosophila* *Jung-Wan Mok*[1,2], Kwang-Wook Choi[1] 1) KAIST (Korea Advanced Institute of Science & Technology), Daejeon, South Korea; 2) Department of Molecular and Human genetics, Baylor College of Medicine, Houston, TX

Proper regulation of cell proliferation and cell death is essential for tissue homeostasis and organ growth in developing animals. Multiple evolutionarily conserved signaling pathways regulate the growth of tissues and organs. Hippo signaling is a conserved mechanism for controlling organ growth. Increasing evidence suggests that Hippo signaling is modulated by various cellular factors for normal development and tumorigenesis. Hence, identification of these factors is pivotal for understanding the mechanism for the regulation of Hippo signaling. *Drosophila* Mnat9 is a putative N-acetyltransferase that is required for cell survival by affecting JNK signaling. Here we show that Mnat9 is involved in the negative regulation of Hippo signaling. RNAi knockdown of Mnat9 in the eye disc suppresses the rough eye phenotype of overexpressing Crumbs (Crb), an upstream factor of the Hippo pathway. Conversely, *Mnat9 RNAi* enhances the eye phenotype caused by overexpressing Expanded (Ex) or Warts (Wts) that acts downstream to Crb. Similar genetic interactions between *Mnat9* and Hippo pathway genes are found in the wing. The reduced wing phenotype of *Mnat9 RNAi* is suppressed by overexpression of Yorkie (Yki), while it is suppressed by knockdown of Hippo upstream factors like Ex, Merlin, or Kibra. Mnat9 co-immunoprecipitates with Mer, implying their function in a protein complex. Furthermore, Mnat9 overexpression together with Hpo knockdown causes tumorous overgrowth in the abdomen. Our data suggest that Mnat9 is required for organ growth and can induce tumorous growth by negatively regulating the Hippo signaling pathway. It has been suggested that Mnat9 functions in developing *Drosophila* are evolutionarily conserved since loss-of-function phenotypes of Mnat9 can be effectively rescued by expressing human homolog protein hNAT9. Our data also indicate that Mnat9 and hNAT9 share similar features in inducing tumor growth through Hippo signaling interaction. As the biological function of hNAT9 in humans are currently unknown, these results are expected to provide essential information on the role of hNAT9 in humans and how it relates to the Hippo signaling pathway.

725V Transcriptomic and functional analysis of a larval brain tumor in Drosophila *Victoria Mendiz*[1], Cayetano Gonzalez[1,2] 1) Institute for Research in Biomedicine (IRB Barcelona), The Barcelona Institute of Science and Technology; Carrer Baldiri Reixac, 10, 08028 Barcelona, Spain. ; 2) Institucio Catalana de Recerca i Estudis Avançats (ICREA); Pg Lluis Companys 23, 08010 Barcelona, Spain.

Mutation on the gene *brain tumour*(*brat*) results in malignant neoplasms that originate from the typeII neuroblast lineage [1–4]. To identify the key oncogenic processes involved in brat tumour development we have combined a large-scale genetic screen to identify genes that can be targeted to inhibit tumour growth (brat-SPRs) and RNAseq transcriptome profiling. We have identified 75 brat-SPRs among 4000 randomly selected genes. Highly enriched among them are proteins involved in translation and vesicle trafficking. We have also found a total of 282 genes that are significantly dysregulated in the tumour. Our results show that upregulation of gene expression correlates poorly with functional requirement: only 5% of the genes that are most highly upregulated in brat tumours are brat-SPRs. Likewise, only 35% of all brat-SPRs identified are significantly upregulated in brat tumours. The same conclusions were derived from an earlier similar study on tumours caused by mutation in lethal(3)malignant brain tumour (l(3)mbt)[5], that are equally malignant, but originate from the neuroepithelium and present notably distinct transcriptomic signatures. Notably, there are only 20 genes in common between the 75 brat-SPRs and the reported 131 mbt-SPRs[5]thus showing that most of the suppressors of tumor growth are tumour-type specific.

726V Regulation of early wing disc growth by Dilp8 *Jeffrey Bellah*, Cora Bergantiños, Albana Kodra, Laura Johnston Department of Genetics and Development, Columbia University Medical Center, New York, NY

The relaxin-like secreted peptide, Drosophila insulin-like peptide 8 (Dilp8), was first identified as a regulator of the delayed pupariation induced by neoplastic or damaged imaginal discs. Through its receptor leucine-rich repeat-containing G protein-coupled receptor 3 (Lgr3) in a pair of bilateral central brain neurons, Dilp8 represses the activity of PTTH neurons, leading to a delay in the surge of the steroid hormone Ecdysone that stimulates pupariation. In addition, Dilp8 and Lgr3 are required for intra- and inter-organ growth regulation, in which damage to discs can slow the growth of undamaged regions and other discs.
To deconstruct how Dilp8 contributes to wing disc growth during normal disc development we examined its expression and that of *lgr3* in whole larvae and wing discs during the 2nd and 3rd instars. We find that relative to whole larvae, *dilp8* is very highly expressed in early 3rd instar wing discs. However, by late 3rd instar *dilp8* expression is considerably decreased in both, in a temporal pattern that is reciprocal to Ecdysone receptor activity in the wing disc. We compared wing discs from wildtype (WT) and *dilp8* mutant larvae across the 2nd and 3rd instars, and find that in *dilp8* mutants wing discs are initially smaller than WT discs at the early 3rd instar, but reach a similar size as WT discs by late 3rd instar. These data suggest that Dilp8 regulates the kinetics of wing disc growth during normal development. Dilp8 overexpression has been shown to modulate Ecdysone, Juvenile Hormone and insulin/insulin-like growth factor signaling, suggesting Dilp8 may regulate these pathways under physiological growth conditions. We aim to determine whether and how normal developmental changes in hormonal signaling mediate Dilp8's role in tissue growth.

727V Influence of B chromosomes on gene expression in the *D. melanogaster* germline *Paulo Belato*, Kaylah Samuelson, Stacey Hanlon Department of Molecular and Cell Biology, University of Connecticut, Storrs, CT

B chromosomes are supernumerary genetic elements found in hundreds of species across a wide range of taxa. Even though many B chromosomes are present in low copy numbers (1-2), the B chromosomes recently discovered in a stock of *Drosophila melanogaster* are carried at a relatively high copy number of 10-12 B chromosomes. Though these B chromosomes do not appear to carry active genes and are mostly heterochromatin, wild-type females with B chromosomes exhibit a significantly increased frequency of Chromosome 4 nondisjunction, leading us to speculate that the presence of B chromosomes may have an influence on other important meiotic processes. We set out to investigate the impact of these B chromosomes on transcription of protein coding genes during gametogenesis in both males and females by performing RNAseq on mRNA extracted from whole testes and the tips of ovaries (germarium through stage 6). Unfortunately, the original B chromosome stock carries a null mutation of *matrimony* (*mtrm*^{126}), a critical regulator of Polo kinase during female meiosis, held over the balancer chromosome *TM3, Sb Ser*, making it difficult to assign significant transcriptional changes due solely to the B chromosomes since a genotypically comparable stock does not exist. To overcome this obstacle, we outcrossed the original B chromosome stock to a *Pr Dr/TM3, Sb Ser* stock that does not carry B chromosomes to create two new stocks, both with B chromosomes and either *Pr Dr* or *mtrm*^{126} held over the *TM3, Sb Ser* balancer. Together with the *Pr Dr/TM3, Sb Ser* stock without B chromosomes, these three genotypes allow us to triangulate genes that are differentially expressed in the germline due to the presence of these B chromosomes and not because of other confounding genetic factors. Our transcriptome data is currently being analyzed as part of a collaboration with the Cabral De Mello Lab from São Paulo University, and we hypothesize that the gene expression of mechanical and regulatory proteins involved in chromosome segregation, such as microtubules, kinetochore proteins, and Bub kinases, will be elevated in response to the presence of supernumerary B chromosomes. We anticipate that our RNAseq analysis will serve as the foundation for new investigations in the genetic pathways that are disrupted due to the presence of B chromosomes during *Drosophila* gametogenesis.

728V Variation in genomic instability due to heat stress in early and late meiosis: Regulation of transcription and chromatin availability *Ulku Altindag*[1], Ruksana Amin[1], Natalia Rincon[1], Brianna Stanley[2], Rita Graze[1], Laurie Stevison[1] 1) Auburn University; 2) University of Alabama at Huntsville

Changes in the environment affect organisms in several aspects including the cellular, population, and ecosystem levels. At higher levels, populations are affected by changes in fecundity and longevity, whereas at molecular levels organisms can be impacted at the genetic level leading to heritable variation if changes are in the germline. Genomic instability results from the increased stress response in multiple pathways, including during meiosis, regulation of chromatin, and DNA repair. In this study, the primary aim was to investigate mechanisms of genomic instability by assessing the relationships between chromatin availability, DNA damage, and differential gene expression. Two species were used to compare, the model system (*Drosophila melanogaster,* DMEL) to a species (*Drosophila pseudoobscura,* DPSE) that is more vulnerable to changes in environmental temperatures. Species-specific treatment crosses were set up at appropriate temperature ranges (standard vs high temperature), early and late meiotic stages were compared by assessing ovaries dissected from females before and after the first mature eggs developed. RNA from the ovaries was used to assay differential gene expression, pinpointing expression differences during early and late phases of meiotic events of pachytene checkpoint, double-strand break formation, and eggshell chorion assembly. Moreover, the ovaries were used to assay apoptosis to measure the DNA damage using DAPI staining to observe the effect of heat stress. Lastly, nuclei from ovaries were extracted to generate ATAC-seq libraries to investigate changes in chromatin availability in each treatment and time point. Results from this study allow for the comparison between species with different thermal tolerances and provide a mechanistic explanation of the relationship between stress response and genomic instability in the context of meiotic recombination.

729V Active site phosphorylation of CDK11 is antagonised by PNUTS-PP1 and localised in the centrosomes *Abdulrahman Aljabri*[1,2], A Campbell [1], D Byrne [1], P Eyers [1], C Eyers [1], D Bennett [1] 1) Institute of Systems, Molecular and Integrative Biology- University of Liverpool ; 2) College of Pharmacy- Taibah University

Reversible protein phosphorylation is a post-translational modification (PTM) that plays a vital role in the regulation of cellular function and is controlled by protein kinases and phosphatases, which add and remove phosphate respectively. Serine/Threonine Protein Phosphatase 1 (PP1) has multiple and essential roles in cellular processes, including RNA splicing, cell division and metabolism of glycogen (Cohen, 2002). In vivo, PP1 catalytic activity is regulated by PP1 interacting proteins (PIPs), one of the most abundant of which is PP1 Nuclear Target Subunit (PNUTS). The PNUTS-PP1 holoenzyme has been implicated in many cellular functions including proliferation, DNA repair, and cell cycle progression and mRNA synthesis. To better understand the role of PNUTS/PP1 we recently conducted phosphoproteomics analysis to identify additional substrates of *Drosophila* PNUTS/PP1. We found that PNUTS/PP1 associates with and regulates the phosphorylation state of the protein kinase Pitslre/CDK11. CDK11 becomes hyper-phosphorylated in its active site at S712 (or S538 in a shorter isoform) when binding of PP1 to PNUTS is disrupted by a mutation in the PP1-binding site of PNUTS (W726A). S712 in CDK11 is equivalent to a serine residue in CDK7 that modulates CDK7 activity and binding to

cyclin H (Larochelle et al., 2001; Martinez et al., 1997) (Endicott & Noble, 2013).
Our current focus is therefore to determine when and where CDK11 is phosphorylated. In *Drosophila* neuroblasts we found elevation of phosphorylated CDK11 (pCDK11) during mitosis, and enrichment of pCDK11 staining in centromeres. Overexpression of kinase dead forms of CDK11 resulted in short or aberrant mitotic spindles . To investigate in vivo function further, we generated non-phosphorylatable (S712/538A) and phosphomimic (S712/538E) CDK11 alleles by CRISPR-Cas9 mediated mutagenesis. We found that non-phosphorylatable CDK11 is recessive lethal in early larval stages, indicating that CDK11 phosphorylation is likely to play an essential role.
Here we will describe the results of our findings and discuss the possibility that pCDK11 modulates CDK11 activity during the cell cycle to promote mitotic spindle formation or function.

730V A non-cell-autonomous buffering mechanism protects cells from replication stress-driven DNA damage Benjamin Boumard[1], *Tania Maalouf*[1], Marwa El Hajj[1], Marine Stefanutti[1], Gwenn Le Meur[1], Reinhard Bauer[2], Allison Bardin[1] 1) Institut Curie, PSL Research University, CNRS UMR 3215, INSERM U934, Stem Cells and Tissue Homeostasis Group, Paris, France.; 2) Molecular Developmental Biology Unit, Life & Medical Sciences Institute (LIMES), University of Bonn, Carl-Troll-Straße 31, 53115 Bonn, Germany.

Replication stress is an important driver of genome instability in cancer. It can be triggered by imbalances in the dNTP pool, which is a common strategy of chemotherapeutic drugs. With the aim of understanding the effects of induced replication stress on stem and progenitor cells, we characterized the consequences of depletion of dNTPs in *Drosophila* adult intestinal stem cells and developing wing disc progenitors. Our findings suggest a previously unrecognized buffering mechanism of the dNTP pool from neighboring cells that depends on Gap Junctions. Specifically, the knock-down in intestinal stem cells of *RnRL*, a subunit of Ribonucleotide reductase - a rate-limiting enzyme essential for dNTP production, leads to high levels of DNA damage, S phase arrest, and ultimate depletion of stem cells from the intestine. In contrast, the clonal knockdown of *RnRL* in wing disc progenitor cells leads to little to no DNA damage. However, when Gap Junctions are inactivated along with *RnrL*, high levels of DNA damage are now induced in the wing disc. This suggests that wing disc progenitor cells participate in dNTP-resource sharing through connections to their neighboring cells via Gap Junctions allowing buffering of induced replication stress. We find that in the intestine, Gap Junctions form between mature enterocytes, whereas intestinal stem cells lack them. Intestinal stem cells, therefore, cannot buffer their dNTP pool via neighboring cells, likely contributing to their exquisite sensitivity to induced replication stress. Overall, our data suggest that a shared dNTP pool can buffer replication stress and that differences in Gap Junction connectivity between cell types may have an important impact on DNA damage susceptibility. These findings have significant implications for understanding replication stress in healthy tissues and cancers.

731V REV7 Acts Independently of Polymerase ζ to Maintain Genome Stability During Development *Lara Maggs*, Mitch McVey Tufts University, Medford, MA

Genome stability is of vital importance during an organism's life. Without appropriate and timely DNA damage response, development is misregulated or entirely disrupted, leading to unhealthy or non-viable offspring. Recent mammalian work indicates REV7 plays a significant role in DNA damage response. REV7 is a component of Polymerase ζ, used in translesion DNA synthesis (TLS), as well as influences double-strand break (DSB) repair pathway choice as a member of the shieldin complex. REV7, a HORMA domain protein, employs its stereotypical safety belt region to interact with other proteins in these complexes.

As such, we wanted to investigate whether REV7 has functions beyond TLS in Drosophila. We used mutant stocks in which the REV7 coding sequence is interrupted by a piggyBac transposable element to characterize phenotypes during normal development and after treatment with exogenous DNA damaging agents. Our studies indicate REV7 loss impairs development to adulthood, decreases hatching rate, and produces abnormal eggshells. The sub-Mendelian ratio of homozygous flies indicates the importance of REV7 in early development. This conclusion is further bolstered by decreased hatching rates, reinforcing the significance of REV7 during development in response to innate endogenous damage. Imperative to successful development is the eggshell, a sophisticated proteinaceous structure composed of chorion, produced en masse via gene amplification. rev7 mutant females produce eggs with partially translucent eggshells. This phenotype has been observed in other DSB repair mutants and is attributed to persistent damage resulting from replication fork collisions during amplification at two chorion loci. Data collected from REV3, Pol ζ's catalytic subunit, mutants do not echo REV7 loss, suggesting REV7 is acting independently of Pol ζ.

Our findings are consistent with REV7 acting in additional non-TLS roles. We are currently working to identify protein partners that interact with REV7 in support of genome stability. These investigations will provide additional insight into the mechanism(s) by which Drosophila REV7 promotes genome stability in the developmental context.

732V Maintenance of genomic integrity in the male germline of *Drosophila melanogaster* *Kate Lemons*, Kent Golic University of Utah

Successful transmission of genetic information from one generation to the next requires germline stem cells to maintain their genomic integrity. Germ cells with DNA damage, such as double strand breaks (DSBs), are largely eliminated. The pathway that works to eliminate cells containing DNA damage has been well studied in the soma. The upstream protein kinase Chk2 phosphorylates P53 which leads to apoptosis. However, in the germline, Chk2 is necessary for elimination of germline cells while P53 is not. This leads to the question: what is downstream of Chk2 in the germline? We used FLP/FRT to efficiently induce DSBs in the male germline. We find that mutation of any of Chk2's domains, including its kinase function, eliminate its DSB checkpoint function in the germline. String (*stg*), a positive regulator of cell cycle progression, is a known target of Chk2 in some systems. If Chk2 negatively regulated Stg in response to a DSB, we reasoned that overexpression of *stg*$^{+}$ might bypass the Chk2 effect and mimic the effect of *chk2* mutants. We found this to be true, strongly suggesting that Chk2 acts through Stg in the germline to block cell cycle progression, ultimately leading to the elimination of cells with damaged DNA.

733V Shared functions of p53 and Xrp1 in DDR and cell competition *Chaitali Khan*[1,2], Nicholas E Baker[1] 1) Department of Genetics, Albert Einstein College of Medicine, Bronx, NY 10461 ; 2) National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD 20892

Cell competition is a process of eliminating relatively unfit cells from chimeric tissues. The tumor suppressor gene p53 is implicated in many instances of cell competition in mammals. There is little evidence for this in *Drosophila,* despite its importance as a model for cell competition. Moreover, mammalian p53 is activated in response to ribosomal defects, whereas *Drosophila* p53 does not seem to be. We hypothesized in *Drosophila* a downstream target of p53 carries out some of its functions and additionally receives inputs from cellular stresses that *Drosophila* p53 does not recognize. This could be the transcription factor Xrp1, a known target of p53, which unlike p53 is activated in response to ribosomal defects and is a key effector of cell competition. Our gene expression analysis shows that Xrp1 is upregulated downstream of p53 in response to acute DNA damage and regulates the expression of molecular players of DNA repair pathway, oxidative response, Jun-kinase signaling and partly the pro-apoptotic gene *hid*. Further, we show that Xrp1 is necessary and sufficient for the expression of several of p53 target genes, but, we find that Xrp1 is only partly needed for DNA damage-induced cell death. Importantly, by activating p53 in a cell non-lethal manner, we show that differences in p53 levels can cause the elimination of cells with higher p53 when growing next to the cells with lower levels of p53. We perform the two-clone assay to confirm that cells with higher p53 are eliminated by cell competition and that this could occurs by activating Xrp1. Interestingly, removing Xrp1 function to show its role in eliminating cells with higher p53 revealed that Xrp1 not only mediate p53 dependent cell competition but is also needed to restrict the clonal expansion of p53 mutant cells. Which we explained by establishing that Xrp1 acts in a p53- independent manner to limit the passage of aneuploid cells created by DNA damage, via its role in cell competition. This further supports the idea that cell competition eliminates aneuploid cells. Overall, these studies establish a role of the p53 pathway in cell competition in *Drosophila* as well as mammals, with Xrp1 as a mediator of acute DDR function, cell competition, and genome stability.

734V Loss of *rer1*-mediated ER-stress drives cell competition in the developing *Drosophila* wing epithelium *Pranab Kumar Paul*, Varun Chaudhary Indian Institute of Science Education and Research (IISER) Bhopal, India

Metazoan development is a robust, error-prone process that is tightly orchestrated by several intrinsic mechanisms. Cell competition is one such process where the defective cell is sensed and eventually eliminated from the tissue. The removal process of defective cells is crucial in the course of healthy tissue development, and several mechanisms of cell competition have been extensively studied to reveal the fact. Using *Drosophila* as a model, many genes have been identified which can provide both selective advantages and disadvantages to the suboptimal cells, but the sensing events caused due to the physiological changes inside an unfit cell before the initiation of elimination are not conserved and still poorly understood.
Here, using the wing imaginal disc of *Drosophila*, we establish that the loss of *rer1*, an essential regulator of ER homeostasis and Notch pathway activity in higher organisms, can trigger cell competition during the development of the polarised wing discs epithelium. In the absence of Rer1, cells are eliminated through cell death via the upregulation of Unfolded Protein Response (UPR) in ER. Moreover, the elimination of *rer1* depleted tissue is blocked by overexpression of the growth arrest and DNA damage-inducible protein (Gadd34), a derepressor of global translation machinery, within the mutant tissue. Unexpectedly, we found that rer1 mutant tissue showed no significant reduction in the level of protein synthesis despite having high levels of P-eIF2α, an integrated stress reporter and repressor of protein synthesis machinery, indicating that P-eIF2α may play a role other than blocking translation machinery in the absence of Rer1. However, if the excessive ER stress or any unknown reason for increased P-eIF2α in *rer1* depleted tissue to trigger the elimination of mutant cells is yet to be investigated further.

735V Regional differences in timing of apical cell area change associated with sex comb rotation during development nicolas Malagon, *Anari-Mai Byfield*, Eleanor Reimer, Stephen Ingram, David Nelson Canadian Mennonite University

A sex comb is a row of bristles present on the first leg of *Drosophila melanogaster* males. During development, these

bristles rotate from a perpendicular to a parallel position on the long axis of the leg. In previous work, we quantified the changes in apical cell area in 4 regions using 5-cell clusters surrounding the sex comb and found those 4 regions display irregular oscillations in size, increase and decreasing in apical cell area. To understand the cell dynamics underlying these observations we studied the entire region located above the comb (30 cells) during the period between 23 and 36 hours after pupation (AP). Using timelapse videos of confocal micrographs of the development of the forelegs of ubi*D*E-Cadherin::GFP flies, we visually determined the apical area by tracing the cells in ImageJ. We observed a reduction in variation in apical cell area associated with comb rotation. However, this uniformity in size is achieved following highly variable local dynamics in apical cell size. This work suggest that irregular oscillations of apical size may be due to local mechanical conditions which underly how overall change in tissue size can occur in a gradual manner.

736C The effects of developmental ethanol exposure on markers of aging in *Drosophila melanogaster* *Navneet Sanghera*, Khaoula Belhorma, Rachael French San Jose State University

Fetal Alcohol Spectrum Disorder (FASD) results from consumption of alcohol during pregnancy, and can lead to a variety of effects including developmental delays, low body weight, and intellectual disabilities. We have established a *Drosophila* model for FASD, and have demonstrated that flies exposed to ethanol during larval development exhibit phenotypes very similar to those seen in mammals. In addition, we recently found that flies reared in ethanol-containing media showed reduced age-related decline in negative geotaxis. In addition, we found persistent ethanol-induced upregulation of the antioxidant genes *Sod1* and *Gss1* in adult flies, continuing for at least 5 days after removal from ethanol-containing food.

Because age-related declines in negative geotaxis are associated with increased levels of oxidative damage to the central nervous system, we hypothesize that increased expression of cellular antioxidants as a result of ethanol exposure during development leads to a decreased ate of aging. To test whether this is a general effect on aging, rather than a specific effect on climbing ability, we are performing several sets of experiments to test the effects of developmental ethanol exposure on cellular and organismal aging. We will present the results of these experiments, including tests of fecundity over time, mortality and lifespan assays, and cellular measurements of the accumulation of oxidative damage.

737A Live longer, climb further: *Parabacteroides distasonis* promotes healthy aging and gut barrier integrity in *Drosophila melanogaster*. *Luana Machado*[1], Sofia Rosa[1], Sarah Shnayder[1], Jim Crott[2], Mitch McVey[1] 1) Tufts University, Medford, MA; 2) Human Nutrition Research Center on Aging, Boston, MA

The term “inflammaging” has been accepted as an all-encompassing term for the age-related increase in inflammation that occurs in organisms. Inflammaging causes many negative impacts on health and lifespan and is associated with cancer progression. Interestingly, some bacteria have been shown to have anti-inflammatory properties. One example is the obligate anaerobe *Parabacteroides distasonis* (Pd), which was recently shown to decrease cancer incidence and progression in a mouse colon cancer model through the modulation of systemic inflammation.
Because inflammation has been linked to aging and decreased health, we explored the effects of Pd treatment on lifespan and healthspan in a Drosophila model system. We found that chronic administration of Pd in the food of adult flies extends the mean lifespan of two genetically distinct strains by 19% and 28% in males and females respectively. In addition, consumption of Pd promotes the maintenance of climbing ability in a negative geotaxis assay, suggesting that Pd also promotes healthy aging.

Having established the impact of Pd on lifespan and healthspan, we began our search for the molecular mechanism through which this bacteria is acting. We first explored its effect on gut membrane stability through Smurf assays, a qualitative approach to examining gut leakage by analyzing how much blue dye diffuses from the intestinal tract into the abdomen of the flies. By comparing dye leakage among treatment groups, we observed that Pd reduces gut barrier defects and increases gut stability in flies as they age.

Given these findings, we hypothesized that Pd could maintain gut membrane integrity in flies by impacting the expression and localization of smooth septate junction proteins (tight junction protein homologs) in the gut, decreasing systemic inflammation, or indirectly preventing hyperproliferation of intestinal stem cells. We conducted q-RT-PCR on candidate genes related to these processes. Our results suggest that expression of inflammatory markers and proteins required for smooth septate junction formation increases with age, but their levels are not affected by treatment with Pd. Analysis using RNA-Seq is ongoing. We are also using fluorescent imaging to visualize potential physical differences among gut tissue of varying treatment groups, specifically looking at stem cell proliferation patterns and smooth septate junction formation. Identification of conserved molecular pathways through which Pd exerts its effects could have therapeutic applications in humans.

738B Developing a quantitative analysis of cysteine availability via iodoTMT-multiplex method using *Drosophila* S2 cells and w^{1118} eyes. Sarah Stanhope Purdue University

Reactive oxygen species (ROS) serve as intracellular signaling molecules; however, in excess ROS molecules are damaging to biomacromolecules such as DNA, lipids, and proteins. The excess accumulation of ROS leads to oxidative stress, disrupting redox homeostasis in the cell and has been linked with aging and neurodegenerative disease. The eye is particularly vulnerable to oxidative stress due to the tissue's high energy demand and generation of ROS by products. In proteins, the thiol group on cysteine residues is susceptible to oxidation. Cysteine residues have multiple oxidation states that can be classified into two categories—reversible and irreversible. Irreversible oxidation states include sulfinic and sulfonic acids, which may alter or impair the activity of the protein depending on the position of the cysteine residue. Understanding how the proteome is affected by increasing ROS levels may provide insights into proteins that are potential targets for oxidation. Here, we sought to evaluate how increasing amounts of oxidative stress altered the redox proteome landscape in *Drosophila.* To characterize the redox proteome, we developed an iodoTMT-multiplex isolation, labeling, and enrichment method to identify cysteine availability and potential oxidation in both S2 cells and w^{1118} fly eyes. To do this we exposed S2 cells to 20mM H_2O_2 and w^{1118} flies to prolonged blue light, followed by redox proteome profiling with iododTMT-sixplex reagents. S2 cell studies revealed 127 significantly oxidized proteins including multiple well-known oxidative stress proteins such as Glutathione S-transferase D1 (GstD1) and Superoxide dismutase [Cu-Zn] (Sod1). GO term analysis elucidated proteins involved in cellular metabolic processes, sulfur compound processes, and cofactor metabolic processes. Further studies in blue light exposed w^{1118} eyes revealed 42 significantly oxidized proteins involved in nucleoside metabolic processes, regulation of cytosolic calcium ion concentration, and glycosyl compound metabolic processes. Interestingly, the overlap of the identified proteins from the S2 cell and w^{1118} studies revealed oxidation of methionine metabolism enzymes suggesting that oxidative stress may perturb methionine metabolism affecting metabolic processes within the cell. As methionine metabolism is critical for lifespan across various organisms understanding how it can be affected by increasing oxidative stress and its role in the eye is critical.

739C Drosophila STING protein has a role in lipid metabolism Katarina Akhmetova, Maxim Balasov, *Igor Chesnokov* University of Alabama at Birmingham

STimulator of INterferon Genes (STING) is an endoplasmic reticulum-associated transmembrane protein that plays an important role in innate immune response by controlling the transcription of many host defense genes. STING has been extensively studied in mammalian immune response, however, the role of STING in the innate immunity of insects have been just recently identified. Immune system is tightly linked with metabolic regulation in all animals, and proper re-distribution of the energy is crucial during immune challenges. Insect fat body integrates these two important systems: it serves as a major immune organ, and also stores excess nutrients (mostly in the form of lipids) and mobilizes them during metabolic shifts. In this work we describe a novel function of Drosophila STING (dSTING) in lipid metabolism. We discovered that flies with a deletion of *dSTING* are sensitive to the starvation and oxidative stress. Detailed analysis revealed that *dSTING* deletion resulted in a significant decrease in the main storage metabolites, such as TAG, trehalose and glycogen. We identified two fatty-acid biosynthesis enzymes - Acetyl-CoA carboxylase (ACC) and fatty acid synthase (FASN) - as the interacting partners for dSTING. Moreover, we found that FASN and ACC interacted with each other, indicating that all three proteins might be components of a large complex. Importantly, *dSTING* deletion leads to the decreased FASN activity and defects in ACC cellular localization suggesting a direct role of dSTING in lipid metabolism of fruit flies.

740A The steroid hormone ecdysone regulates growth rate in response to oxygen availability *George Kapali*[1], Viviane Callier[2], Samuel Gascoigne[3], Jon Harrison[2], Alexander Shingleton[1,3] 1) University of Illinois at Chicago, Chicago, IL; 2) Arizona State University, Tempe, AZ; 3) Lake Forest College, Lake Forest, IL

In almost all animals, physiologically low oxygen (hypoxia) during development slows growth and reduces adult body size. The developmental mechanisms that determine growth under hypoxic conditions are, however, poorly understood. Here we show that the growth and body size response to moderate hypoxia (10% O_2) in *Drosophila melanogaster* is systemically regulated via the steroid hormone ecdysone. Levels of circulating ecdysone are elevated under hypoxia, and inhibition of ecdysone synthesis ameliorates the negative effect of low oxygen on growth rate. We also show that the effect of ecdysone on growth rate under hypoxic conditions is mediated by the insulin/IGF-signaling (IIS) pathway. Hypoxia reduces systemic IIS activity and the hypoxic growth-response is eliminated in larvae with suppressed IIS. Critically, hypoxia transiently increases the expression of *Imp-L2,* an ecdysone-response gene that suppresses systemic IIS. Inhibition of ecdysone synthesis eliminates this increase in *Imp-L2* expression, while loss of Imp-L2 significantly reduces the negative effect of hypoxia on final body size. Collectively, these data indicate that growth suppression in hypoxic *Drosophila* larvae is accomplished by systemic endocrine mechanisms that overlap with mechanisms that regulate growth in response to low nutrition. This suggests the existence of growth-regulatory stress response mechanisms that respond to general environmental perturbation rather than individual environmental factors.

741B Beauty of adenosine and immune system metabolism *Pavla Nedbalová*, Lenka Chodáková, Nikol Kaislerová, Tomáš Doležal University of South Bohemia in České Budějovice

Immune system activation is an energy demanding process requiring metabolic changes on the organismal level

to ensure enough energy for immune cells to successfully face the pathogen. Our laboratory is interested in immunometabolism and uses *Drosophila melanogaster* larvae infected with parasitoid wasp *Leptopilina boulardi* to induce immune system activation and examine both, systemic metabolic changes caused by activated immunity and also metabolism changes of the immune cells themselves.
We have shown that larval immune cells actively produce extracellular adenosine as a systemic metabolism regulator upon infection. Extracellular adenosine is inhibiting glucose uptake by non-immune tissues such as imaginal discs. Thus, there is a high level of glucose available for activated hemocytes. The question is, what is the metabolic origin of this extracellular adenosine in hemocytes?
It is known in mammals that S-adenosylmethionine (SAM) cycle accelerates in activated immune cells. This metabolic pathway is responsible for the methylation of diverse biomolecules and this reaction produces S-adenosylhomocysteine (SAH), a potent inhibitor of this pathway, which is immediately converted to adenosine and homocysteine by S-adenosylhomocysteine hydrolase (Ahcy). Therefore, we have considered Ahcy and SAM cycle as a possible source of extracellular adenosine and we have already obtained data confirming this hypothesis and showing that SAM cycle activity increases also in activated *D. melanogaster* hemocytes.
Our data further imply that adenosine is not only released from hemocytes but is also highly used intracellularly. The first step of the SAM cycle is a unique reaction in which the adenosyl moiety of ATP is consumed along with methionine to synthesize SAM. We propose that a part of the adenosine pool produced by Ahcy is recycled back to ATP through the cooperation of adenosine kinase, adenylate kinase, and glycolysis, as losing all of the adenosine would be a huge energy wasting since all used ATP would have to be refilled by de novo purine synthesis. Our current effort is to examine this complex metabolic network showing adenosine as a crucial molecule affecting not only systemic energetic metabolism and supporting immune response but also participating in the intracellular energy balance of activated immune cells.

742C The *Drosophila* gene *sima* is an essential regulator of the larval glycolytic program Yasaman Heidarian, Liam Mungcal, *Jason Tennessen* Indiana University

The rapid growth that occurs during *Drosophila* larvae requires a dramatic rewiring of central carbon metabolism to support biosynthesis. Previous studies in our lab demonstrated that this exponential growth phase is preceded by a dramatic metabolic switch, which results in the transcriptional up-regulation of genes involved in carbohydrate metabolism, including those that encode enzymes involved in glycolysis and the pentose phosphate pathway as well as Lactate Dehydrogenase. The resulting metabolic program exhibits the hallmark characteristics of aerobic glycolysis and establishes a physiological state that supports biomass accumulation similar to that seen in tumor cells. Studies in the fly have so far have only discovered a single transcription factor involved in this process - the *Drosophila*Estrogen-Related Receptor (dERR), which is absolutely required to up-regulated carbohydrate metabolism in preparation for larval growth. Here we describe our discovery that Sima, the sole *Drosophila* ortholog of the hypoxia inducible factor-1 alpha (Hif1-a), is also essential for promoting aerobic glycolysis in larvae. Using CRISPR/CAS9 generated *sima* alleles that disrupt an exon shared among all isoforms, we discovered that the classic *sima* p-element insertion mutation *sima* ^{kG07607} represents a weak hypomorph that exhibits very mild metabolic defects. In contrast to this canonical allele, our novel null mutations exhibit a mid-L2 lethal phase and phenocopy the metabolic defects observed in *dERR* mutants. Moreover, transcriptomic analysis of *sima* mutants reveal a dramatic downregulation in glycolytic genes, with the overall transcriptional profile mimicking that of the *dERR* mutant. Subsequent metabolomics studies revealed that, when compared to control larvae, *sima* trans-heterozygous mutants exhibit >90% decrease in lactate and 2-HG and a two-fold increase in the circulating disaccharide trehalose. Altogether, these preliminary studies indicate that sima, like dERR, is an essential regulator of the larval metabolic program – a result supported by previous findings that Sima can physically bind dERR. Our future studies will examine the interaction between Sima and dERR to understand how these two evolutionarily conserved transcription factors control aerobic glycolysis in the context of both normal animal development and cancer metabolism.

743A Investigating the mechanism of the pro-aging effects of blue light in *Drosophila* *Jun Yang*, Yujuan Song, Kelsey Shimoda, Benjamin Ramsell, David Hendrix, Jadwiga Giebultowicz Oregon State University

Blue light (BL) is increasingly present in the human environment since BL-enriched LEDs became one of the most common and inexpensive light sources in our homes and workspaces. Blue light is known to damage retina-related cells but also decrease the lifespan in the nematode, *C. elegans*. Our recent data showed that daily exposure of fruit fly (*Drosophila melanogaster*) to 12-h of blue light per day accelerates their aging process and shortens their lifespan. The lifespan of flies kept in constant blue light decreases even more significantly – a quarter of the lifespan for dark control (20 days maximum). To understand the mechanism of blue light damage, we transferred flies kept in constant blue light for 6-, 10-, 14-, and 16-days to a constant-dark environment. Mortality curves suggest the blue light damage could be reversed after 14- but not 16-days of BL- exposure. Next, we measured the gene expression with RNA-seq sampled from heads of 6-, 10-, and 14-day-BL-exposed and age-matched dark control flies. DESeq2 gene expression analysis revealed extensive transcriptome re-programming in response to blue light. Over thousands of genes were significantly altered with several genes strongly upregulated, including transcription factor *Xrp1*, small heat shock protein *l(2)*

efl and *Arc1*. Differentially expressed genes were enriched for functional groups such as neuropeptides, immunity, and metabolism. To further study the metabolic processes affected by blue light, we performed metabolomics using LC-MS and GC-MS. We detected 216 metabolites in the heads of 10- and 14-day-BL-exposed and control flies. MetaboAnalyst identified 42 compounds significantly altered by blue light. Pathway analysis showed that blue light most significantly impacted compounds in the tricarboxylic acid cycle (TCA), Riboflavin, Propanoate, and Butanoate metabolism. Levels of 4 metabolites in the TCA were reduced while levels of succinate were significantly increased in blue light. Moreover, we also determined that succinate dehydrogenase (SDH) activity was reduced in BL-exposed flies, which suggests the impairment of the electron transport chain in mitochondria. In addition, riboflavin, the precursor of SDH cofactor FAD, was significantly reduced by blue light. Lastly, ADP levels were significantly increased in BL-exposed flies while ATP was decreased. These results show that TCA and mitochondria are impaired in blue light. Another metabolite significantly reduced in BL-exposed flies is glutamate, which serves as an energy substrate and also functions as an excitatory neurotransmitter, and a precursor for the inhibitory neurotransmitter GABA. Taken together, our data suggest that blue light impairs energy metabolism and interferes with brain functions in flies.

744B Nutrient-dependent acyl-CoA metabolism regulates tissue remodeling by adjusting stem cell quiescence and activation in *Drosophila* *Xiaotong Li*, Jason Karpac Department of Molecular and Cellular Medicine, Texas A&M University, College of Medicine, Bryan, TX

Nutrient availability is a major selective force in the evolution of metazoa, and organisms thus need to carefully adapt to nutrient changes in order to promote somatic maintenance and reproduction. This adaptation has also shaped the cellular and molecular mechanisms that underly nutrient-dependent remodeling of tissue function and morphology. Here, we leveraged a meta-analysis of transcriptomics datasets and phylogenomics and discovered that Acbp6 (Acyl-CoA binding protein 6) is a critical regulator of nutrient-dependent tissue remodeling in *Drosophila*. Acbp6 arose by evolutionary duplication in the *Drosophila* order, and is uniquely expressed in functionally differentiated enterocytes of the regenerating fly midgut (intestine). We found that Acbp6, which binds Acyl-CoA with high-affinity and thus dictates cellular Acetyl-CoA metabolism, is required for intestinal regrowth after nutrient deprivation (fasting) through adjusting intestinal stem cell (ISC) activation from quiescence. Fasting induces intestine morphological recession, while refeeding promotes intestinal remodeling and growth in *Drosophila*. Acbp6 expression is induced by fasting, and normalizes after refeeding. However, attenuation of Acbp6 led to abnormal tissue remodeling after fasting, due to uncoordinated regulation of ISC activation and proliferation. Moreover, Acbp6 inhibition also drives aberrant 'switching' of metabolic cycles in enterocytes during nutrient adaptation, which overall impaired Acetyl-CoA production. Decreases in Acetyl-CoA led to attenuation of pan-acetylation of proteins in enterocytes during refeeding and intestinal regrowth. Re-expression of Acbp6 or acetate supplementation can restore pan-acetylation and promote ISC activation from quiescence during nutrient adaptation. Through genetic screening we identified Stat92e, which can be acetylated, as a key regulator of ISC activation from quiescence during refeeding. In enterocytes, Stat92e can govern Upd3 induction during nutrient adaptation, and Upd3 can dictate ISC proliferation and intestinal regrowth. Our data thus define a new regulatory mechanism, shaped by Acyl-CoA metabolism, that is required to adjust stem cell quiescence and activation, and therefore drive tissue remodeling during nutrient adaptation.

745C Endocrine signals from the gut that regulate metabolism *Nadja Ahrentløv*, Stanislav Nagy, Olga Kubrak, Alina Malita, Takashi Koyama, Michael Texada, Kim Rewitz Section for Cell and Neurobiology, Department of Biology, Copenhagen University, Denmark

Maintaining metabolic homeostasis requires interorgan coordination and thus communication, mediated by endocrine signals. Organs with specialized functions sense changes in internal and external nutrient availability and release hormonal factors that coordinate energy intake, storage, and expenditure. In both flies and mammals, the gut, the largest endocrine organ and the first that encounters food after ingestion, is a source of many secreted signals that regulate metabolism. Human gut-derived hormones such as glucagon-like peptide 1 (GLP-1), which regulates both metabolism and appetite, are of great therapeutic value in the treatment of diabetes and obesity. GLP-1 promotes insulin secretion and underlies the "incretin effect," in which ingested glucose induces a larger insulin release than glucose injected directly into the bloodstream, thus bypassing the gut. Insulin action is opposed by the peptide hormone glucagon, but gut hormones that regulate glucagon release are poorly defined.

Many proteins are predicted to be secreted, but the functions of most of these potential hormones are largely unknown; these factors thus represent a significant unexploited resource for the discovery of new endocrine signals from the intestine that affect metabolic balance. Like its mammalian analog, the small intestine, the *Drosophila* midgut contains enteroendocrine cells (EECs) that release a variety of known endocrine factors in response to nutritional quantity and quality. We have performed a large-scale EEC-specific *in-vivo* RNAi-based screen of secreted factors in adult flies with the goal of identifying gut-derived factors that regulate food intake and metabolism. Our phenotypic readouts included measures of male and female flies' ability to survive during nutritional deprivation and their storage of energy (as lipids and glycogen). Among our hits we have found several gut-derived hormonal factors that play key roles in regulating

metabolism and feeding behavior, some of which act in a sexually dimorphic manner by modulating the glucagon analog Adipokinetic hormone. Taken together our results identify several gut hormones that may be of relevance to the treatment of diabetes and obesity.

746A The loss of function mutation in the *Drosophila Neprilysin Like 15* changes expression of key enzymes involved in glycogen homeostasis, and effects longevity in sex specific manner, but exerts similar effects on motor activity in both sexes *Nicolas Jones*, Raghad Almotairy, Thanh Ha Vy Nguyen, Hannah Halmes, Erin Hatcher, Brianna Villines , Surya Banerjee Arkansas Tech University

Obesity predisposes a person to an array of life-threatening metabolic disorders including diabetes, high blood pressure, heart attack, and cancer. The long-term treatment for such diseases is often less effective in providing a complete cure, and the cost for it creates excessive economic burden. Thus, new and more effective therapeutic approaches are under review. In the last two decades, Neprilysins (Neps), the membrane bound metallo-ectopeptidases, have become targets to treat obesity and related disorders for their correlation with high fat and high sugar diet induced obesity and diabetes. Thus, the mechanisms by which Neps regulates obesity need to be explored. In our recent published work, we show that knock-out of the *Drosophila* specific gene *Neprilysin like 15 (Nepl15^{ko})*, which has overall higher expression in the wild type adult male compared to female flies, results in significant reduction of stored glycogen and lipids only in adult males, but increases glycogen in females. Although, the mutant flies show similar food intake compared to the controls. To further dissect the effects on the expression of the key enzymes in glycogen and lipid homeostasis pathway, we recently find that *Nepl15^{ko}* adult males but not females have significant reduction in the expression of Glycogen Synthase and Glycogen Phosphorylase transcripts compared to controls. In addition, we have performed longevity and climbing assay to investigate the effect of Nepl15 loss of function on overall health and physiological motor activity. We reveal that the *Nepl15^{ko}* adult males have similar life span, but *Nepl15^{ko}* females show significantly extended life span compared to the controls. Both *Nepl15^{ko}* adult males and females flies show enhanced ability of climbing in an age dependent manner compared to the controls. For example, whereas there is no difference in climbing ability between the mutant and control males and females up to 30 days of age, the mutant flies of both sexes exhibit significantly higher ability for climbing at the age of 40 days. Thus, our research reveals that mutation in the *Nepl15* provides health benefits in females, and keeps the physical activeness for longer in both sexes due to changes in nutrient homeostasis. In the future, we will investigate the lipid homeostasis pathway and energy metabolism in these flies. This project is funded by Arkansas NIH-INBRE grant.

747B Two phases of ageing in mice, a mammal model for Smurfness. *Celine Cansell*[1], Fanny Bain[1], Vivien Goepp[2], Nicolas Todd[3], Veronique Douard[4], Flaminia Zane[1], Clara Sanchez[5], Nicolas Pietrancosta[6,7], Carole Rovere[5], Raphael GP Denis[8,9], Serge Luquet[9], Michael Rera[1] 1) Center for Research and Interdisciplinarity (CRI), Inserm U1284, Université de Paris, F-75006 Paris, France; 2) MinesParisTech, CBIO – Centre for Computational Biology, PSL Research University, 75006, Paris, France & Institut Curie, PSL Research University, 75005, Paris, France & Inserm, U900, Paris; 3) Centre Roland Mousnier, CNRS, Sorbonne Université, 75005 Paris, France & Laboratory of Population Health, Max Planck Institute for Demographic Research, 18057 Rostock, Germany; 4) Université Paris-Saclay, INRAE, AgroParisTech, MICALIS Institute, 78350 Jouy-en-Josas, France; 5) Université Côte d'Azur, IPMC-CNRS UMR 7275, F-06560 Valbonne, France; 6) Laboratoire des Biomolécules, LBM, Département de chimie, École Normale Supérieure, PSL University, Sorbonne Université, CNRS, Paris 75005, France; 7) Neuroscience Paris Seine-Institut de Biologie Paris Seine (NPS-IBPS) INSERM, CNRS, Sorbonne Université, Paris 75005, France; 8) Université de Paris, Institut Cochin, INSERM, CNRS, F-75014 PARIS, France; 9) Université de Paris, BFA, UMR 8251, CNRS, F-75013 Paris, France

The "Smurf" model of ageing, first described in Drosophila, is characterized by two successive and necessary phases. A first phase A where individuals have no risk of mortality but an age-dependent increasing risk of entering phase B. A second phase B where individuals show a high risk of impending death from natural causes. Individuals in phase B show typical hallmarks of ageing such as decreased energy storage, locomotor activity and fertility, a deregulation of insulin signaling, and a strong increase in systemic inflammation. The discovery of this model was made possible by a singular characteristic of phase B, its increased intestinal permeability. To assess intestinal permeability in Drosophila, a non-absorbable and non-toxic blue food dye is added to the medium of adult individuals. In contrast to phase A, for individuals in phase B, the dye crosses the intestinal epithelium and reaches the hemolymph turning individuals blue. According to their color, blue individuals were hence dubbed "Smurfs".
We propose here for the first time that the two phases of ageing separated by the "Smurf" transition are relevant to mice, thanks to a longitudinal longevity study using two different mouse strains, in both males and females. By integrating physiological, metabolic and molecular measurements with the life history, we assessed the predictive power of the model concerning the high risk of impending death in this model organism. This allows us to answer three research questions:
(1) As described in Drosophila, is the end of life in mammals characterized by an increase in intestinal permeability?
(2) Is there a specific physiological signature of the end of life in mammals?
(3) Do identified biomarkers allow us to discriminate between two subpopulations characterized by different mortality

risk at any chronological age?
The characterization of this “Smurf” two-phase ageing model in mammals is essential to study and to better understand ageing because it allows a primordial thing currently still impossible: to evaluate the physiological age of individuals. It also allows a better understanding of the underlying mechanisms of ageing by offering the possibility to exclude individuals presenting the classically accepted hallmarks of ageing in order to define the causes of the appearance of these hallmarks. Finally, it is a putative game changer in the domain of personalized medicine paving the way for a unifying and truly public model of ageing, while extending the Drosophila model to mammals.

748C Investigating Flock House virus-mediated changes in bioenergetics in aged *Drosophila melanogaster* *Dean Bunnell*[1], Eli Hagedorn[1], Beate Henschel[2], Daniel L. Smith[3], Stephanie Dickinson[2], Andrew W. Brown[2], Maria De Luca[3], Ashley Turner[4], Stanislava Chtarbanova[1] 1) University of Alabama; 2) Indiana University; 3) University of Alabama at Birmingham; 4) Jacksonville State University

We have previously shown that Flock House Virus (FHV) kills older flies more rapidly than young adults without higher virus titers. This indicates that survival to FHV infection depends on tolerance mechanisms, which are not well characterized. FHV triggers profound transcriptomic changes in older *Drosophila* at time points before significant mortality is observed in young and aged cohorts. The age-dependent impairment of disease tolerance to FHV in *Drosophila* is characterized by stronger regulation of genes whose products function in mitochondria and mitochondrial respiration. We hypothesized that the *Drosophila* bioenergetic profile will be deregulated following FHV infection. The first 3 days of infection, we performed whole-organism respirometry of 5- and 30-days old individual *Drosophila* receiving non-injected, Tris-injected (control) or FHV-injected treatments to determine if metabolism plays a role in infection outcomes. Pairwise comparisons were conducted on the Least Squares Mean Oxygen Consumption Rate (OCR) to detect statistically significant differences between treatment groups. Our results show that FHV infection significantly reduces OCR compared to non-injected ($p=0.0264$) and Tris-injected ($p=0.0164$) controls. Although the factor of «Age» and the interaction of «Age x Treatment» did not have a significant effect on OCR, the interaction of «Age x Time Post-Treatment» shows a significant change in OCR in aged flies compared to young individuals at 24-hours. The OCR signature at 24-hours varied in response to both treatment and an individual’s survival status at 48-hours. FHV-injected flies that died prior to 48-hour measurements had a significantly lower OCR ($p=0.0438$) compared to flies that were alive at 48-hours.

749A Coordinated shifts in redox metabolites during quiescence are heritable factors that reprogram progeny metabolism *Helin Hocaoglu*, Lei Wang, Mengye Yang, Sibiao Yue, Matthew Sieber UT Southwestern Medical Center, Dallas, TX

Maternal diet and metabolic stress have a profound effect on health and disease susceptibility of progeny. Previous studies have shown this transgenerational effect; however, the mechanisms remain unknown because of the challenges in obtaining pure populations of quiescent oocytes. To overcome this challenge, we utilized the *Drosophila* oogenesis system to isolate large quantities of staged oocytes to study how the quiescent nature of oocytes impacts the effect of maternal metabolic stress on progeny metabolism. Using biochemical and systems-based approaches, we found that in the late stages of oogenesis, oocytes acquire a unique redox state due to suppression of mitochondrial oxidative metabolism in a process called mitochondria respiratory quiescence (MRQ). We have found that maternal metabolic stress triggers MRQ prematurely and induces the reprogramming of progeny metabolism and life-long changes in glucose and triglyceride homeostasis. In addition, we have found that maternal metabolic stress causes a disruption in the unique redox state of oocytes by impairing NAD biosynthesis. We have shown that reducing the levels of NAD in the oocyte are sufficient to induce progeny reprogramming, moreover maternal NAD precursor supplementation can suppress the progeny phenotypes caused by maternal metabolic stress. Compromised NAD levels inherited from the oocyte causes impaired methionine cycle activity and a 50% decrease in the production of the methyl-donor S-adenosyl methionine (SAM). Lower levels of SAM lead to a global 30% reduction in H3K27-me3, a corresponding increase in H3K27-ac, and de-repression of 550 genes during embryogenesis. Interestingly, 1/3 of these genes are specifically expressed in the intestine and are involved in processes such as lipid digestion, uptake, storage, protein digestion, nutrient transport, and calcium signaling. As a result of this intestinal metabolic shift, reprogrammed progeny were able to develop better on a low nutrient diet but have a shorter adult life span compared to control progeny. Taken together we believe that progeny metabolic reprogramming is an adaptive tradeoff that tunes progeny metabolism to promote development in a poor nutrient environment, at the expense of a shorter lifespan.

750B Experimental Evolution to identify genes that contribute to fitness in high-sugar-fed Drosophila melanogaster *Thomas Rundell*, Azva Alvi, Melina Brunelli, Christina Capobianco, Gabrielle Safian, Laura Musselman 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 will use genetic approaches to test whether these genes mitigate overnutrition phenotypes, potentially revealing a novel link between the central nervous system and metabolism.

751C Lactate and glycerol-3-phosphate metabolism cooperatively regulate larval growth in a tissue nonautonomous manner *Madhulika Rai*, Hongde Li, Sarah Carter, Maria Sterrett, Geetanjali Chawla, Jason M. Tennessen Indiana University Bloomington

The dramatic growth that occurs during *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. Central to the biosynthetic potential of aerobic glycolysis is lactate dehydrogenase (LDH), which promotes glycolytic flux by regenerating NAD^+. We have seen that although *Ldh* mutants accumulate elevated NADH levels, larvae compensate for this metabolic insult by increasing glycerol-3-phosphate (G3P) production, which serves as a backup mechanism to regenerate NAD^+, and the cooperative regulation of lactate and G3P metabolism imparts metabolic robustness on the larval glycolytic program. Further, lack of *Ldh* and *Gpdh1* together, exhibit developmental delays, synthetic lethality, and aberrant carbohydrate metabolism. Although we understand the effect of the loss of *Ldh* and *Gpdh1* in the whole body, tissue-specific roles of both enzymes remain unexplored. To address this deficiency, we used RNAi to understand how tissue-specific depletion of *Ldh* and *Gpdh1* affects larval growth and metabolism. Our results demonstrate that while individual loss of either *Ldh* or *Gpdh1* in fat body, muscle and neurons does not affect larval development, loss of both *Ldh* and *Gpdh1* within either the fat body, muscle or neurons leads 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 is capable of inducing systemic signals that coordinate intercellular metabolic states with growth of the entire organism. Finally, our preliminary findings indicate the cytokine Upd3 is a key signal in connecting loss of Ldh/Gpdh1 activity in individual tissues with systemic growth delays. Overall, our findings hint at a mechanism that coordinates larval growth with the rate of glycolytic flux in individual tissues.

752A Investigating the role of Glycerol-3-phosphate dehydrogenase 1 (GPDH1) in *Drosophila* growth and development *Shefali Shefali*, Madhulika Rai, Sarah Carter, Nader Mahmoudzadeh, Hongde Li, Maria Sterrett, Jason Tennessen Department of Biology, Indiana University Bloomington, Bloomington, IN, USA

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 allows 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 of these earlier studies is that GPDH1 promotes mitochondrial energy production and triglyceride accumulation while also serving a key role in maintaining cellular redox balance. Moreover, GPDH1 activity is regulated in a stage-specific manner, suggesting that this enzyme plays an essential role in coordinating metabolism with developmental progression. Here we extend these earlier observations using metabolomics to expand upon these earlier observations. Our studies reveal that *Gpdh1* mutants exhibit severe defects in amino acid metabolism, with both the maternal and zygotic GPDH1 enzyme pools influencing amino acid abundance. Moreover, our preliminary studies indicate that GPDH1 indirectly regulates growth signaling in larval tissues by controlling abundance of dihydroxyacetone phosphate. Overall, our results reiterate the importance of GPDH1 activity in larval development and reveal how this enzyme can control growth by previously unappreciated mechanisms.

753B Ribosomal profiling Reveals Changes in the Translatome of *kdm5*-Knockdown Neurons *Matanel Yheskel*, Julie Secombe Albert Einstein College of Medicine

KDM5 is a histone lysine demethylase responsible for the demethylation of H3K4me3, a histone modification associated with transcriptionally active promoters. Mutations in three of the four KDM5 paralogs in humans (KDM5A-C) are associated with intellectual disability but KDM5's role in regulating neuronal function is unknown. RNA-Sequencing of neuron-specific *kdm5* knockdown flies reveals that the majority of ribosome protein genes (RPG) are downregulated compared to control, however there is no dramatic change to bulk translation or translation initiation. Changes in RP stoichiometry has been shown to affect translation efficiency (TE) of different classes of mRNA; to that end, we performed ribosomal profiling in *kdm5* knockdown neurons. This analysis revealed that mRNAs encoding proteins

with roles in glycolysis, the tri-carboxylic acid (TCA) cycle, and fatty acid metabolism showed significantly reduced TE in *kdm5* knockdown neurons. Interestingly, this included genes whose human homologs are implicated in neurological disorders. These data reveal a novel role of KDM5 in the translational regulation of key metabolic pathways necessary for proper neuronal homeostasis and function.

754C Investigating the mechanisms that control glycolytic gene expression at the cessation of larval growth *Tess Fasteen*, Jason Tennessen Indiana University

All growth during the *Drosophila* life cycle is restricted to larval development, when animals increase their body size ~200-fold over the course of four days. In order to support this exponential growth rate, larvae up-regulate glycolytic metabolism as a means to generate the necessary energy and biomass. The resulting metabolic program exhibits the hallmark characteristics of the Warburg effect, or aerobic glycolysis, which is also used by many types of cancer cells to fuel tumor growth. Thus, our ability to study the mechanism that controls larval glycolytic metabolism provides a powerful genetic model to explore how aerobic glycolysis is regulated *in vivo*. In this regard, the fly provides an unusual opportunity to identify the endogenous mechanisms that turn off aerobic glycolysis *in vivo* - unlike tumors, where the Warburg effect is indefinitely activated, fly larvae down-regulate glycolysis at the end of the growth phase in a predictable manner. Using a candidate gene approach, we are exploring two mechanisms that potentially control the global down-regulation of glycolytic gene expression at the onset of metamorphosis. First, we are exploring the possibility that the late-larval pulse of 20-hydroxyecdysone (20E) is necessary to downregulate glycolytic gene expression. Our preliminary studies support this hypothesis, as two metabolite markers of aerobic glycolysis, lactate and 2-hydroxyglutarate, fail to be downregulated in larvae lacking 20E signaling. In addition, we are examining the role of the *Drosophila* Estrogen Related Receptor (dERR) in this late-larval metabolic transition. Since dERR is required to activate glycolytic gene expression at the onset of larval development, and previous studies demonstrate that dERR activity is down-regulated during the late-L3 stage, we are testing the hypothesis that ectopic dERR activation would prevent down-regulation of glycolytic gene expression. Here too, our preliminary data indicate that ERR signaling may need to be attenuated to allow for the downregulation of glycolysis prior to metamorphosis. Overall, our findings suggest that nuclear receptor signaling plays a key role in the transcriptional down-regulation of glycolytic genes at the cessation of larval growth.

755A Mutational characterization of phosphorylation sites suggests sex-specific regulation of the metabolic regulator Lipin *Michael Lehmann*, Madeline Richards, Stephanie Hood University of Arkansas

Lipins constitute a conserved family of eukaryotic metabolic regulators. They are dual-functions proteins, acting as phosphatidate phosphatases in lipid synthesis in the cytoplasm and as transcriptional regulators of metabolism in the nucleus. The single *Drosophila* Lipin ortholog contains 26 serine and threonine phosphorylation sites that were identified by mass spectrometry, suggesting complex posttranslational control of Lipin functions. Indeed, in both flies and mammals, nuclear translocation of Lipin has been shown to be controlled by TORC1-dependent phosphorylation. To identify additional functions of phosphorylation, we have carried out a systematic mutational analysis of single phosphorylation sites and groups of sites, replacing serine and threonine residues with alanine residues or the phosphomimetic glutamic acid. CRISPR/Cas9-generated mutants were then characterized for Lipin expression, metabolic defects, and starvation resistance. Notably, we found that one of these mutants, carrying an amino acid substitution at serine residue 820 (S820), exhibited a significant difference in starvation resistance in the male and female sex. Interestingly, S820 is strategically located between the catalytic and transcriptional co-regulator motifs of the protein. Both males and females expressing Lipin S820E, in which the serine residue is replaced by phosphomimetic glutamate, showed reduced starvation resistance. In contrast, female flies expressing non-phosphorylatable Lipin S820A displayed significantly increased starvation resistance, whereas males showed reduced starvation resistance. These data suggest that the phosphorylation status at specific sites of the Lipin protein differentially affects functions of the protein in males and females. Thus, it may contribute to sex-specific differences in metabolism. The metabolic consequences of S820 phosphorylation in males and females are currently under investigation.

756B Developmental Effects of Cactus on *Drosophila mettleri* *Lidane Noronha*, Brian Lazzaro, Patrick O'Grady Cornell University

The cactophilic *Drosophila* system of the Sonoran Desert is an excellent model not only to study how organisms may thrive in toxic environments, but also how some taxa are associated with the microbes necessary to decompose plant tissue. Cacti contain toxic compounds that protect them from herbivory. When a cactus is injured, sap exudes from its wounds and microorganisms quickly colonize the sap and begin the decomposition process. Interestingly, several *Drosophila* species can exploit these substrates as feeding and breeding sites.
While many members of the cactophilic *Drosophila repleta* species group have been able to transition seamlessly onto a standard cornmeal diet, *Drosophila mettleri*, is an exception. This is the only known species to breed exclusively in soil soaked with necrotic cactus sap and tissue, allowing it to take advantage of a more toxic and microbially rich niche. This taxon requires cactus-supplemented media to maintain healthy cultures under laboratory conditions. We hypothesized

that D. mettleri's reliance on its host plant and associated microbiome resulted in the loss of its ability to adapt to a novel, more sterile environment.
Our understanding of the physiological processes required for *Drosophila* to utilize decomposing cactus as a host plant is limited. For example, we currently do not know whether *D. mettleri* derives nutritional benefit from the decomposing cactus, the saprophytic microorganisms responsible for the decomposition process, or a combination of the two. This study examines *D. mettleri* to understand how various life stages perform when reared on (1) standard media, (2) standard media + cactus powder, and (3) standard media + sterilized cactus powder. Development rates between each life stage and adult feeding behavior were examined. The results show that cactus has an essential role in ensuring that *Drosophila* survive the pupation and eclose successfully.

757C Drosophila Undigested Metabolite Profiling - Uncovering age-related changes in amino acid absorption *Abigail Mornement*, Rachael Dack, Rebecca Clark Department of Biosciences, Durham University, UK

Unintentional weight loss is a common characteristic of ageing and frailty. While this may be partially explained by dietary changes and the metabolic cost of immune activation and inflammation that occur with age, other factors must contribute.
Nutrient absorption is a key role of the intestine which has not been studied in the context of ageing. Given the myriad changes occurring with age in the intestine, from increased bacterial load, to dysplasia and loss of intestinal barrier integrity, we feel that nutrient absorption is a likely contributing factor to this unintentional weight loss.
We have developed an assay to assess changes in the nutrient absorption/excretion profile in response to variables such as age or microbiota composition. Through this, we have found that more nutrients are egested with age, indicating a reduced absorptive capacity which may contribute to the unintentional weight loss observed with ageing and frailty. We are investigating changes in transporter expression with age as a possible mechanism. Additionally, by comparing the profile from axenic and conventionally reared flies, our assay confirms that the microbiota is a large source of protein and suggests that the microbiota further reduces the absorptive capacity of Drosophila. Thus potentially implicating the microbiome in age-related weight loss.

758A Embryonic lipid transport works with TORC1 to ensure rapid and efficient development *Marcus Kilwein*, Michael Welte University of Rochester

Maternal nutrient stores are abundant and transient, providing embryos with energy that fuels development and carbon scaffolds for anabolism. Among this nutrient supply, the fat storage organelles, lipid droplets (LDs), occupy a unique position. At cellularization, most LDs are allocated to the incipient epithelium, while carbohydrate and protein reserves are confined to the yolk cell. Previous work has shown that two distinct mechanisms ensure this privileged localization of LDs. First, the LD protein Jabba keeps LDs and glycogen granules apart; LDs lacking Jabba protein embed into the surface of glycogen granules which results in them being mislocalized to the yolk cell. Second, during the blastoderm stages, LDs move bidirectionally along microtubules, driven by kinesin-1 and cytoplasmic dynein. In the absence of the motor regulator Klar, dynein activity predominates, also resulting in LD mislocalization to the yolk cell. To investigate why this LD distribution exists, we followed *Jabba* and *klar* embryos through subsequent embryogenesis and assessed triglyceride consumption, LD localization, and timing of embryogenesis. The yolk cell localized LDs in *Jabba* and *klar* persisted through hatching, demonstrating that proper LD localization is crucial to their consumption. This disruption in LD consumption appears to have detrimental consequences for the embryo. On the one hand, knocking down the triglyceride lipase Brummer in *Jabba* and *klar* mutants reduces embryo hatching success; under the same conditions, wild type embryos hatch normally. To test whether the hatching delay results from reduced LD consumption or excess LDs damaging the yolk cell, we generated embryos with a reduced maternal supply of LDs using mutants in the LD protein dPLIN2 or in the lipid uptake receptors LpR1 and LpR2. Both genotypes showed significant developmental delays in reciprocal crosses. We therefore conclude embryos respond to reduced access to LD stores with a developmental delay. To elucidate the genetic players in the delay, a candidate RNAi screen was done in *Jabba* and *klar* backgrounds. While, in a wild type background, suppression of mTORC1 signaling resulted in reduction in embryo hatching. Surprisingly, this reduction in viability was partially alleviated in *Jabba* and *klar* mutants potentially indicating that mTORC1 growth signaling needs to be turned down in a lipid-deprived state. This work demonstrates that organization of LDs in embryos works in concert with metabolism machinery to ensure optimal embryonic development.

759B Consequences to Organismal Physiology upon Dysregulation of Hormonal Homeostasis using *Drosophila melanogaster* *Cameron Dixon*, Julia Ye, Kim McCall Boston University

Hormones orchestrate vital physiological pathways, such as metabolism, development, and reproduction. Beyond their influence on organismal homeostasis, hormones maintain integrity of stem cell niches and tissue integrity through molecular mechanisms. The presence of hormones is not restrictive to mammals and is evolutionarily conserved across species down to microorganisms. This conservation through evolution shows the importance in understanding how hormones interact with physiology and the negative implications of what happens to homeostasis post-hormonal disruption. To this end, we will be using *Drosophila melanogaster* to better understand how dysregulation of hormonal

homeostasis impacts physiology. *Drosophila* possess hormonal regulation that shares many similarities with mammalian hormonal homeostasis. While mammals are more complex organisms, many of the vital hormones essential to physiology are conserved. For example, the mammalian peptide hormone, insulin, is present in *Drosophila.* In *Drosophila*, it is known as Drosophila Insulin-like Peptide (DILP) and there are eight forms. Similar to how mammals have various insulin types, these eight forms serve specific functions across the organism to regulate glucose needs and metabolism. Additionally, DILPs are heavily involved in other physiological processes, including other hormonal pathways that regulate development and reproduction. The major hormones that are intimately connected to DILP are ecdysone and juvenile hormone (JH); which function similarly to that of mammalian sex steroids and growth hormones, respectively. All three of these major hormones are not only involved in their own canonical pathways but also influence each other by being necessary for each other to complete their function. To fully understand how these hormones effect physiology once homeostasis is dysregulated, we will be examining the effects of hormonal perturbations on physiology. Perturbations will be completed using the Gal4-UAS system and will be visualized using microscopy methods. Preliminary results have shown consequences to physiology through malformation of wings and increased wing venation, decreased survival, temperature sensitivity, and apoptotic phenotypes in reproductive tissues. Further investigation into these consequences are currently being completed to understand the effects of perturbing major hormones (DILP, ecdysone, JH) and their effects on physiological homeostasis and hormonal crosstalk.

760C Hormonal Effects of Glyphosate Based Herbicides on *Drosophila melanogaster* *Maggie Santos*, Becky Tayln
California State University San Bernardino

Glyphosate based herbicides are the most commonly used world-wide. The effects of glyphosate on reproductive and endocrine systems has not been fully explored. We used a JH inhibitor, precocene, and a JH analog, methoprene, to determine if glyphosate interacts with the JH pathway. So far, the lowest survivability to day three has been for Roundup Super Concentrate treated with precocene. Glyphosate, the active ingredient in Roundup, is a non-selective herbicide. It is the most widely used pesticide due to its indiscriminate ability to kill weeds. Originally believed to only affect plants because it affects the shikimate pathway, which animals lack, studies indicate that glyphosate based herbicides (GBHs) harm animals and microorganisms. GBHs can cause endocrine disrupting behaviors in non-target organisms (Gasnier et al. 2009), including oocyte production (Talyn et al. 2020). We seek to identify a mechanism by which GBHs reduce oocyte production and decrease reproduction, using the Drosophila melanogaster model system. We will use a JH inhibitor, precocene, and a JH analogue, methoprene, along with treatments of GHBs and glyphosate alone to determine whether GBHs act on reproductive output through a pathway involving JH, an important hormone in Drosophila reproduction (Wilson 1982).

761A Distinct dietary nutrients regulate circulating levels of Dilp2 and Dilp6 in *Drosophila* larvae *Miyuki Suzawa*, Dalton Hilovsky, Kyle McPherson, Michelle Bland University of Virginia, Charlottesville, VA

The insulin/IGF1 signaling pathway regulates cell and organismal growth in animals ranging from fruit flies to humans. Insulin-like hormones are secreted in response to dietary nutrients and drive anabolic metabolism. In *Drosophila*, seven insulin-like peptides (Dilps) activate the insulin/IGF signaling pathway through a single known insulin receptor. We previously reported that Toll signaling in *Drosophila* larval fat body inhibited whole-animal growth by reducing circulating levels of fat body-derived Dilp6. In contrast, Dilp2 secretion from insulin producing cells was not affected by Toll pathway activation in larval fat body. To determine whether other physiological conditions differently regulated these two related hormones, we asked how Dilp2 and Dilp6 respond to starvation and specific dietary nutrients. 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 led to a 50% reduction in circulating Dilp2 after 6 hours and a 90% reduction in Dilp2 after 24 hours. In contrast, circulating Dilp6 levels were unchanged over the course of 24 hours of starvation on agar. Next, we asked whether Dilp2 and Dilp6 respond to specific dietary nutrients. We fed larvae sucrose, peptone or yeast extract in agar and compared hormone levels with fed and starved conditions. Hemolymph Dilp2 was partially recovered by feeding larvae peptone or yeast extract but not sucrose. Circulating levels of Dilp6 were modestly increased by a sucrose-only diet, but surprisingly, peptone or yeast extract feeding repressed Dilp6. The reduced circulating level of Dilp6 caused by the high protein, yeast extract diet was partially rescued when sucrose was added back. These data suggest that Dilp2 and Dilp6 secretion are regulated differently by environmental conditions ranging from infection to dietary composition. Two open questions are how Dilp2 and Dilp6 secretion are regulated by specific nutrients and how the single known insulin receptor integrates divergent signals from distinct Dilps to control growth and metabolism.

762B Dynamic expression of Lgr1 in the hindgut suggests a role in cold tolerance and acclimation *Daniel Munteanu*, Sara Helms Cahan University of Vermont

Thermal tolerance of an organism depends on both its ability to dynamically adjust to a thermal stress and preparatory processes that enhance resistance to extreme temperatures (either cold or hot). However, our understanding of the molecular mechanisms that underpin these acclimatization processes is limited—especially for the cold. It has been previously shown that cold acclimated flies are better able to maintain ion homeostasis and fluid reabsorption across

the hindgut epithelium in extreme cold. We had previously conducted a GWAS analysis of the critical thermal minimum (CTmin) in the Drosophila Genetic Reference Panel (DGRP), which identified two single nucleotide polymorphisms (SNPs) in the first intron of Lgr1, a a G-protein coupled receptor (GPCR) expressed predominantly in the hindgut. Baseline Lgr1 expression was higher in flies raised at colder temperatures as adults, suggesting that changes in Lgr1-mediated signaling may be involved in conferring cold stress resistance. To test this hypothesis, expression of Lgr1 was knocked down with Gal4 driving RNAi expression in hindgut. Lgr1 knockdown significantly impaired baseline CTmin in both sexes, and in males, which express ~6X higher Lgr1 under baseline conditions, knockdown reduced cold acclimation capacity. Altogether, this suggests that Lgr1 may play an important role in driving hindgut reabsorption during cold stress. Future studies will focus on elucidating Lgr1 interactors and identifying its part in the preparatory physiological processes changing to maintain homeostasis in the extreme cold.

763C ***Mechanisms of Action and Natural Variation within Fasting-induced Starvation Resistance in*** **Drosophila** *Benedict Lenhart*, Alan Bergland University of Virginia

Nutrient availability is a component of environment that impacts metabolism and organismal fitness. How nutrient-limitation affects organismal survival remains unclear, in part because we do not understand the extent of genetic variation in metabolic response to this environment. Research has established that limiting nutritional availability via a periodic fasting treatment produces health benefits including longevity and starvation resistance across a broad range of taxa, though this work has commonly avoided assaying natural populations. To expand our understanding of how clines such as space and time impact an organism's physiological response to a nutrient-limiting environment, we are establishing a study system from environmentally-distinct populations of *Drosophila melanogaster.* We will sample and create lineages from latitudinally and seasonally varying populations, and leverage the genetic differences between these populations to explore variance in the ability of periodic fasting to improve starvation resistance. In the forthcoming assays: flies will be removed from nutrients for varying extents during early adult life, and their subsequent starvation resistance, fat storage levels, and fat storage dynamics measured throughout adult life. Early results lead us to hypothesize that while three weeks of periodic fasting can improve starvation resistance, this effect is line specific and subject to variation. Moreover, while measurements of fat concentrations will decrease post fasting, fasted-flies will be able to utilize existing reserves with higher efficiency during starvation. This work aims to indicate how schedules of nutrient limitations lead to changes to starvation resistance across natural populations, and how mechanisms of fat storage and lipophagy mediate this variation.

764A Time-restricted feeding improves striated muscle in genetic-induced obese Drosophila *Yiming Guo*[1], Christopher Livelo[1], Shweta Varshney[2], Farah Abou Daya[1], Hiep Le[3], Satchidananda Panda[3], Girish Melkani[1,2] 1) Department of Pathology, Division of Molecular and Cellular Pathology, School of Medicine, The University of Alabama at Birmingham, AL 35294, USA; 2) Department of Biology and Molecular Biology Institute, San Diego State University San Diego, CA 92182, USA; 3) Regulatory Biology Laboratory, Salk Institute for Biological Studies, La Jolla, CA 92037, USA.

Pathological obesity due to genetic predisposition affects countless people globally and leads to several ailments such as cardiovascular disease, metabolic syndrome and impaired muscle function. Our lab has developed a novel genetic-induced obesity model using *Drosophila melanogaster* which contains a *sphingosine kinase 2 (Sk2)* gene mutation. *Sk2* mutation leads to accumulation of ceramide and eventually results in lipotoxicity which hampers muscle function and exhibits obese related metabolic parameters. Interestingly, by employing time-restricted feeding (TRF), an intervention which maintains isocaloric consumption that limits the daily feeding window from ZT0-ZT12, *Drosophila melanogaster* (fruit fly model) with genetic induced-obesity showed attenuated phenotypes. Flies under TRF improved skeletal muscle function, increased insulin sensitivity, reduced intramuscular lipid infiltration and preserved mitochondrial integrity compared to their ad libitum feeding (ALF) counterparts (Villanueva et al, 2019, *Nature Communication*). In this study, we explored the pathophysiological basis of TRF-mediated benefits utilizing transcriptomic data of indirect flight muscle (IFM). We investigated physiology using functional and genetic validations, cytological and biochemical approaches. We found flies under TRF intervention displayed significant upregulation of *Gnmt*, *Sardh* and *CG5955*, key players of *S-adenosylmethionine* regulation, and downregulation of *Dgat2*, a key gene for triglyceride synthesis. IFM-specific knock-down (KD) of *Gnmt*, *Sardh* and *CG5955* led to progressive muscle dysfunction. Further, IFM-specific KD of *Dgat2* retained muscle function during aging. In addition, genes associated with AMP kinase (AMPK) signaling, glycogen metabolism, glycolysis, tricarboxylic acid (TCA) cycle and electron transport chain (ETC) signaling were specifically upregulated in GIO model under TRF. Altogether, we identify the GIO-specific pathways in the regulation of muscle function under TRF, which emphasized the role of feeding-fasting rhythms on combating comorbidities linked with genetic obesity. Validation of these genes in obesity-induced skeletal muscle health and the role of circadian clock in that process is currently underway.

765B Time-restricted feeding promotes skeletal muscle function in diet-induced obesity through purine related pathway in Drosophila *Christopher Livelo*[1], Yiming Guo[1], Shweta Varshney[2], Farah Abou Daya[1], Hiep Le[2], Satchidananda Panda[2], Girish Melkani[1] 1) University of Alabama at Birmingham School of Medicine; 2) Salk Institute for Biological studies

Millions globally are affected by obesity stemming from a lifestyle of calorie-dense diets. Consequently, individuals afflicted with diet-induced obesity (DIO) incur complications including metabolic syndrome, cardiovascular disease, and compromised muscle function. In a previous study, we employed a time-restricted feeding (TRF) intervention, where daily feeding was limited to only the first 12 hours of the day in a *Drosophila melanogaster* model. Upon administration of a high-fat diet while under TRF, we observed improved skeletal muscle function compared to ad libitum feeding (ALF) counterparts in addition to improved metabolic parameters such as insulin sensitivity, mitochondrial integrity and body mass (Villanueva et al 2019, *Nature communication*). In this study, we evaluate the mechanistic basis of TRF-mediated benefits in DIO by utilizing muscle transcriptomic data of indirect flight muscle (IFM) followed by genetic validations, cytological and biochemical evidence. Interestingly, we found significant upregulation of glycine N methyltransferase (*Gnmt*), sarcosine dehydrogenase (*Sardh*) and *CG5955*, key players of one carbon-metabolism and *S-adenosylmethionine* regulation under TRF. We found downregulation of diacylglycerol o-acyltransferase 2 (*Dgat2*), a key gene for triglyceride synthesis. RNAi knockdown of the upregulated genes led to muscle dysfunction and abrogated TRF-mediated improvement of muscle performance. However, knockdown of *Dgat2* preserved muscle performance during aging. Furthermore, de novo purine biosynthesis appeared to be upregulated and potentially led to increased ATP levels resulting in improved muscle performance. Folic acid, a key factor required in fueling the purine cycle was found to attenuate high-fat diet related muscle impairment. These findings contribute to potential mechanistic foundations that underlie TRF mediated improvement in muscle and provide a blueprint for pursuing therapy related research development relevant to obesity and muscle impairment.

766C General anesthetics are toxic to flies mutant for a mitochondrially-encoded subunit of the electron transport chain. *Amanda Scharenbrock*, Zachariah Olufs, David Wassarman, Misha Perouansky University of Wisconsin-Madison, Madison, WI

Animals with mutations in Complex I of the mitochondrial electron transport chain (mETC) exhibit behavioral sensitivity to volatile general anesthetics (VGAs) and may be at increased risk of VGA-induced deleterious collateral effects. To investigate the toxicity of VGAs in mitochondrial mutants, we studied flies carrying a mutation in *ND2*, a gene in the mitochondrial genome that encodes a protein in the intermembrane arm of Complex I. We examined two commonly used VGAs isoflurane (ISO) and sevoflurane (SEVO), halogenated ethers, as well as various oxygen (O_2) concentrations. ISO proved to be toxic in hypoxic (5% O_2), normoxic, and hyperoxic (75% O_2) conditions while SEVO was only toxic in hyperoxic conditions. Interestingly, our previous work has shown that flies carrying a mutation in a nuclearly-encoded Complex I subunit, *ND23*, die when exposed to ISO in normoxia and hyperoxia, but ISO in hypoxia and SEVO under any oxygen conditions were not toxic. We have also expanded our anesthetics to include halothane (HALO), an alkane anesthetic, which like SEVO is non-pungent. Unlike SEVO, we found that HALO is toxic to both *ND2* and *ND23* mutants, with greater toxicity in *ND2* mutants. These data indicate that mutations in different subunits of Complex I of the mETC confer differential susceptibility to VGA-induced toxicity. To investigate the underlying mechanism, we have begun examining whether heterozygous mutations in candidate genes enhance or suppress VGA-induced toxicity in *ND2* mutants. To date, we have found that mutation of *sima*, which encodes a subunit of Hypoxia Inducible Factor (HIF) that transcriptionally regulates the response to hypoxia, enhanced the lethality of *ND2* mutants exposed to ISO under normoxic or hypoxic conditions, indicating that HIF functions to prevent VGA toxicity. This work is part of an ongoing project aimed at identifying modifiers of VGA toxicity in mitochondrial mutants.

767A What Ingredients are Contributing to the Toxicity of Glyphosate-Based Herbicides, in Drosophila melanogaster? *Noelle Roddam*, Kalinah Winston, Becky Talyn California State University- San Bernardino

Growing evidence shows that some herbicides, including those intended to target plants, can be toxic to non-target animals and even humans, and that toxin residues remain in our food and water and pass to us through consumption. Our previous work, focused on glyphosate-based Roundup® formulations, indicates that Drosophila melanogaster fruit flies are a good model species to study these toxic effects on animals, by investigating their behavior, reproduction, anatomy, development, and mortality. This present study compares the toxicity of commercial glyphosate-based herbicide formulations to the toxicity of their key ingredients, alone and in specific mixtures. We hope to gain a better understanding of the toxic effects that glyphosate and other key ingredients lend to the toxicity of the formulations they are contained within, as well as that produced by the combination and interaction of the ingredients themselves. Preliminary results indicate that Glyphosate alone is not as toxic as the Roundup® formulations tested, which suggests that other ingredients, such as the known or unknown surfactants, are contributing to the overall toxicity of these formulations.

768B Positive selection of senescence through increased evolvability: ageing is not a by-product of evolution. Tristan Roget[1,2], Pierre Jolivet[3], Sylvie Méléard[1], *Michael Rera*[4] 1) École Polytechnique, Palaiseau, France; 2) Université de Montpellier, Montpellier, France; 3) IRIT-APO - Algorithmes Parallèles et Optimisation - Institut de recherche en informatique de Toulouse, France; 4) Centre de Recherche Interdisciplinaire, INSERM, Université de Paris

The possibility of ageing being directly selected through evolution has been discussed for the past hundred years.

As ageing is occurring, by definition, only late in life, many think that it cannot be actively selected as a process. In addition, by decreasing an individual's fitness, it is thought unlikely to be selected for. In order to explain the observation of its broad presence in the realm of life, numerous theories have been proposed in the past 60 years. Here, building upon a simple two parameters life history trait model that we recently introduced and that summarizes the life of an organism to its two core abilities - reproduce and thrive -, we discuss the possibility of ageing being selected for through evolution.
Our model shows that senescence will appear and be positively selected for through evolution thanks to the higher evolvability it confers to organisms. In addition, it predicts that the Lansing effect should be conserved in a large proportion of organisms showing age-related senescence. This formal and numerical analysis of ageing's evolution also provides new hints to test the validity of existing theories by proposing a simple mathematical interpretation of the long-discussed longevity-fertility tradeoff or mutations accumulation.

769C Smurfness helps deconvolving ageing transcriptional signature *Flaminia Zane*[1,5], Hayet Bouzid[1], Céline Cansell[1], Jean-Michel Camadro[2], Sylvere Durand[3], Christophe Antoniewski[4,5], Michael Rera[1] 1) Center for Research and Interdisciplinarity (CRI), Inserm U1284, Université de Paris, 75006 Paris, France.; 2) Plateforme Protéomique/ Spectrométrie de masse, Institut Jacques-Monod, 75013, Paris, France.; 3) Metabolomics platform, Institute Gustave Roussy, 94 805 Villejuif, France; 4) ARTbio bioinformatic analysis platform, Institute of Biology Paris Seine, Sorbonne Université, F-75005, Paris, France.; 5) Sorbonne Université, 75006, Paris, France

Ageing is a process affecting a broad range of living organisms. In humans and multiple model organisms, it is characterized by an age-dependent decrease in functional efficiency and increased vulnerability to death. The classical approach for studying ageing involves comparing individuals of different chronological ages. Even if deregulation in certain pathways and mechanisms have been identified as associated with ageing, the so-called hallmarks of ageing, a comprehensive picture of the process is still missing. We believe that the inability to look at the physiological age of individuals -at the moment of sampling- rather than their chronological age is an important limiting factor in ageing research.

In 2012, Rera and collaborators described a new age-dependent and death-related phenotype in *Drosophila melanogaster*. By looking at a physiological increase in the intestinal permeability to a blue food dye, they were able to identify individuals committed to death in a few days. Interestingly, these individuals, called "Smurfs", are the only ones, among a population, to exhibit various age-related changes and high-risk of impending death whatever their chronological age; Smurfness has been therefore hypothesized to be a good proxy to follow physiological age of single individuals.

We are here presenting our work on the characterization of the Smurf transcriptome using *D. melanogaster*. Through whole-body RNA-sequencing on Smurf and age-matched non-Smurf flies of different chronological ages, we demonstrate that Smurfs carry a stereotypical transcriptional signature independently of their age. Strikingly, this signature mostly overlaps the previously described transcriptional signature of ageing. By studying concomitantly time-related changes and smurf-related changes in gene expression, we were able to identify genes moving through time but not necessarily associated to the physiological collapse of the organism and death. Those results confirm that not only Smurfs are a valid model to look at the physiological age of *D.melanogaster*, but are a powerful tool for deconvolving the changes related to physiological and chronological time. In conclusion, they highlight the importance of considering smurfness when designing ageing studies.

770A The role of commensal microbes in the longevity effects of Aronia berry (*Aronia melanocarpa*) in *Drosophila melanogaster* *Ji-Hyeon Lee*, Hye-Yeon Lee, Subeen Shin, Kyung-Jin Min Department of Biological Sciences and Bioengineering, Inha University, Incheon, Republic of Korea

Aronia berry (*Aronia melanocarpa*) is rich in polyphenolic compounds with various physiological and pharmacological activities. Several biologically active substances such as flavonoids and phenolic acids, have been reported to increase the lifespan of model organisms. With the increasing interest in the study of gut microbiota, it has been found that gut microbiota plays an important role in host health and aging. Recent studies have shown that the gut microbiota composition is changed by environmental factors like diet and aronia berry can modulate the gut microbial flora and lead to maintain a healthy/balanced gut microbiota. Although many studies have shown that aronia berry has anti-aging and prebiotic effect, the relationship between commensal microbes and the anti-aging effect of aronia berry is not illustrated yet. To test whether the anti-aging effect of aronia berry is related with commensal microbes, we generated axenic flies and measured their lifespan with or without aronia berry supplementation. In addition, 454-pyrosequencing analysis of the 16S rRNA gene was performed to investigate the microbial flora alteration in flies. Our results will provide the fundamental knowledge to address the relationship between commensal microbes and anti-aging effect of aronia berry.

771B The fly Tumor Necrosis Factor Receptor (TNFR), Wengen, restricts cytoplasmic TRAF3 levels to control gut metabolism, immunity, and tissue homeostasis Rihab Loudhaief, Christian Christensen, Julien Colombani, *Ditte*

Andersen University of Copenhagen

The family of tumor necrosis factors (TNFs) are implicated in diverse processes ranging from cell proliferation, differentiation and apoptosis to innate and adaptive immunity. While TNFs contribute to tissue homeostasis and immunity, they are also notorious for their pathological roles in promoting tumor growth and inflammatory diseases. In addition to their well-described pro-inflammatory functions, an increasing body of evidence suggest that TNF signaling plays an instrumental role in orchestrating the metabolic rewiring associated with disease conditions in both flies and mammals. While this suggests that TNF can act as a metabolic hormone, it is not known whether TNF-TNFR signaling regulates to metabolic processes in healthy individuals. Here we show that the fly TNF receptor (TNFR), Wengen (Wgn), localizes in intracellular vesicles in the adult fly gut and plays an important role in controlling lipid metabolism, tissue homeostasis and immunity. Knocking down Wgn in enterocytes (ECs) results in activation of autophagy, degradation of lipids, and increased sensitivity to starvation. Moreover, *wgn* mutant guts display ectopic activation of JNK/MAPK and IMD signaling causing accelerated epithelial turnover and increased immune activity. We further show that all these effects are ligand-independent and mediated by TNF associated factor (TRAF)3, as Wgn is required to restrict cytoplasmic levels of TRAF3. These findings suggest that in addition to previously reported pathological roles of TNF-TNFR signaling in promoting inflammation and disrupting energy homeostasis, TNFRs are crucial mediators of metabolism and tissue homeostasis and moderators of immunity in healthy organisms.

772C Screening for the genetic polymorphism underlying aging-related muscle degeneration *Christina Talley*, Soobin An, Kaveh Kiani, Anton Bryantsev Kennesaw State University

Aging-related muscle tissue decline negatively impacts the quality of life for elderly individuals and causes billions of dollars in healthcare costs. The genetics of this phenomenon is not well understood, although such information may be critical for preventive care. Notably, previous attempts to link genetic polymorphisms with aging-related muscle decline have been limited to muscle structural genes.

We used *Drosophila* TDT (jump) muscle to study aging-dependent loss of muscle fibers. The TDT consists of 20-30 muscle fibers and functions to power jumping in flies. With progressive age, individual TDT fibers undergo spontaneous degeneration that is evident by the loss of cytoplasmic structures and nuclei. The extent and frequency of TDT degeneration are quantifiable and appear to be line dependent.

We have conducted a small genome-wide association analysis using 30 inbred fly lines demonstrating various rates of aging-dependent TDT fiber loss. Our data suggest that genes associated with the functioning of the nervous system are the strongest modifiers of TDT degeneration rates. To validate this finding, we further analyzed TDT degeneration rates in flies with TDTs uncoupled from the nervous system as well as seizure-prone flies with chronically hyperactivated nervous systems.

Our data demonstrates that deviations in the normal functioning of the nervous system significantly influence spontaneous muscle fiber degeneration during aging. Our results can guide future identification of the genetic polymorphism underlying muscle tissue decline in humans.

773A Identifying the regulatory basis of sex differences in reproductive senescence in *Drosophila melanogaster*. *Ruksana Amin*, Caroline Hawkins, Rita Graze Auburn University

Senescence is the gradual decline of function with advancing age. For example, in humans aging increases risk of hearing and vision loss. Reproductive senescence, is specifically the deterioration in reproductive success with advancing age. Hormone signaling pathways such as the insulin and insulin-like growth factor-like signaling (IIS) have many roles in aging and other processes. The IIS pathway, for example, can modulate not only lifespan, but also reproductive output, growth, metabolism, and stress. It has also been suggested that this pathway is likely to play a role in reproductive senescence. However, most reproductive senescence studies have focused on only one sex, though both show reproductive senescence and there are important effects of male-female interactions, Similarly, while the role of the IIS network in aging is well-characterized, there are few studies that have investigated tissue and age specific differences in both sexes allowing a direct comparison of changes in IIS with age. In order to understand why reproductive aging commonly differs between the sexes, we investigated how aging impacts sexually dimorphic expression of reproductive senesence candidate genes in different tissues – including those in the IIS pathway and in the sex determination hierarchy. Somatic and gonadal tissues were collected from males and females in a time series spanning day 3 to day 42 (post eclosion). RNA seq analysis will reveal how males and females differ in expression changes with advancing age.

774B dSmad2 MARCM clones reveal a requirement for dILP2 secretion in the adult brain *Samuel Goldsmith*, Stuart Newfeld School of Life Sciences, Arizona State University, Tempe AZ

Previous studies in our lab of dILP2 in the adult female brain showed that dCORL (fuss in Flybase) was required for

dILP2 expression in a subset of insulin producing cells (IPCs). To test the hypothesis that dCORL is acting downstream of dSmad2[SG(1] in IPCs, as was shown earlier in the mushroom body of third instar larvae, we generated dSmad2 mutant MARCM clones in IPCs. Additionally, a series of follow-up experiments in IPCs were completed and the results were consistent with the data from the dSmad2 mutant MARCM clones. Employing a dCORL.Gal4 driver expressed in IPCs, dSmad2 mutant MARCM clones displayed excess dILP2 expression in comparison to adjacent wild type siblings (n=5; p=0.057). The overexpression phenotype was also seen with dSmad2 RNAi in IPCs driven by dCORL.Gal4 (n=14; p=0.013). The overexpression phenotype was rescued when expressing UAS.dSmad2 in mutant MARCM clones (n=7; p=0.67). The dSmad2 mutant phenotype appears similar to dILP2 overexpression in mutants for unpaired-2 that prevent dILP2 secretion. Studies with TrpA1 to test the secretion hypothesis are in progress. Overall, our study shows that dCORL functions independently of dSmad2 in the regulation of dILP2 expression in adult IPCs.

775C Identification of transcription factors acting in larval fat body to regulate whole-animal growth *Dalton Hilovsky*, Kyle McPherson, Leila Jamali, Shivani Reddy, Michelle Bland University of Virginia, Charlottesville, VA

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. Indeed, our previous work showed that innate immune signaling in larval fat body targets Dilp6 to inhibit peripheral growth. To identify novel regulators of growth, we performed a directed genetic screen targeting transcription factors expressed in larval fat body. We screened for transcription factors that regulate peripheral growth basally and in response to active innate immune signaling in fat body. In a related screen, we queried a set of transcription factors shown to physically interact with the *Dilp6* promoter using data from the ENCODE consortium. These screens identify novel regulators of whole-animal growth such as the transcription factor pleiohomeotic like (phol), which modifies growth inhibition resulting from Toll signaling in larval fat body. Additionally, by screening predicted transcriptional regulators of *Dilp6*, we identified that increased expression of hairy (h) in fat body from the mid-third instar through the white prepupal stage reduces *Dilp6* transcript levels. In contrast, fat body-specific knockdown of daughterless (da) suppresses *Dilp6* expression in mid-third instar larvae but not in white prepupae. These two screens have identified discrete regulators that participate in Toll-dependent growth inhibition and *Dilp6* regulation. Indeed, previous data from our lab indicate that reduced Dilp6 secretion in animals with active fat body Toll signaling does not account for the full degree of growth inhibition in these animals. Furthermore, our data point to additional, unappreciated signaling pathways that contribute to developmentally-appropriate regulation of Dilp6.

776A *Lgr1* Localization Reveals a Larval-to-Adult Developmental Switch in Hindgut Compartmentalization *Luis Sullivan*[1], Robert Scott[1], Rosario Vicidomini[2], Mihaela Serpe[2], Benjamin White[1] 1) Laboratory of Molecular Biology, National Institute of Mental Health, NIH, Bethesda, MD 20892, USA; 2) Section on Cellular Communication, National Institute of Child Health and Human Development, NIH, Bethesda, MD 20892, USA

An evolutionarily ancient hormonal signaling system consisting of two glycoprotein hormone-like subunits, *gpa2* and *gpb5*, and their leucine-rich repeat containing G-protein coupled receptor, called *Lgr1* in *Drosophila,* are found throughout bilaterian genomes from flies to humans. The function of this conserved hormonal system remains obscure, but has been best characterized in the mosquito and the fruit fly, where Lgr1 is strongly expressed in the hindgut and has been implicated in maintenance of hydromineral balance. Using the Trojan exon method to create an Lgr1-specific Gal4 driver, we have confirmed robust expression of Lgr1 in both larval and adult hindgut, as well as in numerous other structures that support chloride transport such as the stellate cells of the Malpighian tubules and larval anal papillae. Interestingly, expression in the larval hindgut is limited to a ventral compartment, whereas in adult hindgut it is expressed throughout the hindgut epithelium. Using scRNAseq analysis combined with gene-specific Trojan Gal4 driver lines, we find that the larval epithelium is compartmentalized into ventral and dorsal cell-types conforming to those that express *Delta* and *engrailed* in the embryonic hindgut. Ventral cells express several genes implicated in ion transport and antidiuresis, while dorsal cells selectively express receptors for two known diuretic hormones, Dh44 and Leucokinin (Lk). These gene expression patterns suggest that half of the larval hindgut may promote water uptake from the hemolymph while the other may promote ion retrieval from the hindgut into the hemolymph. We speculate that this division of labor allows the larva to survive in its quasi-aqueous environment, permitting it to shed absorbed water, but retain ions. Consistent with this, hindgut compartmentalization is lost in the adult hindgut, together with Lk receptor expression, suggesting a purely antidiuretic function in the adult, which has a terrestrial lifestyle in which water retention is paramount.

777B *fruitless* Controls the Timing of Steroid Hormone Pulses in *Drosophila* Somatic Cell *Jie Sun*, Calder Ellsworth, Wu-min Deng Tulane University School of Medicine

Approximately 50% of all cancer patients develop cachexia, a devastating wasting syndrome. This figure increases up to 80% in patients with advanced cancers. In recent years, a number of *Drosophila* cachexia models have been established, however, steroid hormone level changes, a frequent symptom of cachexia in patient, has remained insufficiently

understood. We demonstrate here that the BTB zinc-finger transcription factor *fruitless (fru)* is a key player of steroid hormone signals and negatively regulates the synthesis of ecdysone in prothoracic gland (PG) cells by responding to steroid hormone levels in the body. Specifically, we show that Fru oscillates within the PG cells: a high concentration of Fru in the nuclei is concomitant with low-titer ecdysone levels in the body, and the protein is absent from PG nuclei at developmental stages when high-titer ecdysone pulses occur. Depletion of Fru in the PG accelerates larval development by causing precocious ecdysone signaling and a failure to repress ecdysone pulses. In contrast, excessive Fru expression in the PG arrests development that can be rescued by administrating an active ecdysteroid, and we show that Fru inhibits the ecdysteroid biosynthesis pathway by regulating Halloween genes. Notably, we show that this regulation has implications for the cachexia model. Fru in PG nuclei of cachexic larvae is maintained at a high level, and the periodicity of Fru expression associated with normal development is abolished, thereby maintaining a low level of circulating steroids in cachexic larvae. These findings suggest that aberrant expression of Fru in PG is a major reason for hormone imbalance in cachexic larvae and is partially responsible for the cachexia phenotype, thus provide a theoretical basis for hormone treatment of cachexia.

778C Studying the effect of Methotrexate on DNA damage and repair during ageing: drug treatments and models of JAK/STAT pathway-related blood cancers *Adel Alqarni*, Martin Zeidler University of Sheffield

Originally developed as a chemotherapy drug, MTX acts as an anti-folate by binding to and inhibiting di-hydrofolate reductase (DHFR) that is involved in the synthesis of nucleotides required for DNA damage repair and cellular proliferation. In the 1980's, MTX was repurposed as a treatment for auto-inflammatory and auto-immune diseases such as rheumatoid arthritis at much lower doses. However, the mechanism of action of MTX as an anti-inflammatory agent has been unclear until 2015 when the Zeidler lab identified MTX as a JAK/STAT pathway inhibitor. While our insight suggests that MTX could be repurposed again to treat JAK/STAT associated diseases, concerns about potential mutagenic toxicities associated with its inhibition of DHFR have been raised.
My project uses *Drosophila melanogaster* to study the effects of low-dose MTX therapy, its potential mutagenic activity and its effects on JAK/STAT signaling in vivo. More broadly, I aim to examine the impact of MTX and to better understand the processes that modulate DNA damage and DNA-damage responses.
We have established an *in vivo* assay for DNA damage using *w+; GMR-Gal4, UAS-white* RNAi / + flies to report the frequency of DNA damage in cells of the developing fly eye. Using this assay, along with measures of lifespan, fecundity, chromatinization, and JAK/STAT pathway activity we have tested a range of MTX doses added into *Drosophila* food. The effects on survival, development and DNA damage phenotypes will be shown. My results indicate that that high drug concentrations result in low survival rates, fecundity and developmental defects as well as a significant increase in the rate of DNA damage. However, low doses of MTX do not affect survival or fecundity and surprisingly, show significant increases in lifespan and may even reduce the rate of DNA damage. Suggesting that reduced JAK/STAT pathway activity may be providing a benefit under these conditions.
In conclusion, while high doses of MTX lead to low survival and are mutagenic, low-doses may confer a survival and DNA-damage advantage. It will be interesting to establish whether these low-dose benefits are indeed linked to changes in JAK/STAT pathway activity in vivo. Latest results will be presented.

779A Determining Critical Period of Herbicide Sensitivity in the Fruit Fly, *Drosophila melanogaster* *Becky Talyn*[1], Gabrieila Melchiorre[2], Erik Melchiorre[3] 1) College of Natural Sciences, California State University, San Bernardino; 2) Sage Oak Charter School; 3) Geology Department, California State University, San Bernardino

Herbicides are widely used in agriculture, but many harm animals in addition to weeds. The most widely used brand of herbicide, Roundup®, occurs in multiple formulations, with proprietary ingredients, but currently all Roundup® formulations sold in the U.S. contain the active ingredient glyphosate. In this study, we determine the critical period for *Drosophila melanogaster* in terms of mortality and harm to the reproductive system after exposure to a Roundup® formulation with only glyphosate as an active ingredient, a Roundup® formulation that contains glyphosate and pelargonic acid, or Scythe®, an herbicide that contains only pelargonic acid as an active ingredient. For all herbicide treatments and exposure at all life stages, fewer larvae survived to adulthood than in control treatments at concentrations above 0.5 g/L. Preliminary data suggests that first instar larvae are most susceptible to mortality, regardless of herbicide exposure. Reproductive ability decreased with higher concentrations of herbicide exposure, regardless of formulation or exposure stage, possibly because survival was so low for those exposed starting at their 1st instar. Overall, 1st instar larvae are more susceptible to the harmful effects of exposure to both glyphosate-based and pelargonic acid-based herbicides than those exposed at later stages, and larvae of all stages are more sensitive than adults tested in previous studies with the same formulations and range of concentrations.

780B Retrotransposons: a major driving force of aging *Blair Schneider*[1], Shixiang Sun[1], Moonsook Lee[1], Wenge Li[1], Jay Cadiz[1], Nicholas Skvir[2], Nicola Neretti[2], Jan Vijg[1,3], Julie Secombe[1,4] 1) Department of Genetics, Albert Einstein College of Medicine, Bronx, NY; 2) Department of Molecular Biology, Cell Biology, and Biochemistry, Brown University, Providence, RI; 3) Department of Ophthalmology and Visual Sciences, Albert Einstein College of Medicine, Bronx, NY; 4) Dominick P. Purpura Department of Neuroscience, Albert Einstein College of Medicine, Bronx, NY

Across all metazoans, aging correlates with functional decline. One potential contributor this is the expression and mobilization of retrotransposons (RTs), which are mobile genetic elements capable of self-replication and insertion into new genomic locations. RT expression and copy number have been shown to increase with age within multiple model organisms. Our goal is to determine de novo RT insertion number and genomic location within somatic cells of *Drosophila*. By carrying out whole genome sequencing from individual indirect flight muscle (IFM) nuclei from young and old female flies, RT insertions increase with age, with one of the most age-associated new insertions being from the element 412. However, unlike many RTs, the expression of 412 mRNA does not increase with age but remains constant throughout life. Additionally, this increase in RT insertion with age may not occur in all tissues, as when we purified nuclei from whole female thoraces that reflect a range of distinct cell types, we do not observe an age-associated increase in RT insertions. To determine the biological impact of 412 expression and/or insertion, we ubiquitously knocked down the expression of 412 using an inducible shRNA transgene and CRISPRi. This significantly extended female median and maximum lifespan. However, performance in several assays of health was not improved when 412 was knocked down, indicating that longevity is being targeted irrespective of changes to health. Since, we observe increase in 412 insertions in IFM, we also knocked down 412 specifically in this tissue and saw an increase in female lifespan, consistent with the importance of TE insertions in this cell type. In contrast, lifespan was unaltered by knocking down 412 in fat body. To understand the basis for the increased lifespan caused by reducing 412 expression we carried out RNA-sequencing using thoraces of control and 412 knockdown animals. These data suggest that knockdown flies may have improved mitochondrial function as several components of the electron transport chain are significantly upregulated. We are currently testing if the NADH/NAD and ATP levels differ as one of the classic hallmarks of aging is mitochondrial dysfunction. Importantly, our data highlight that the expression and/or mobilization of RTs is associated with the aging process potentially through a more efficient metabolic process.

781V Parkinson's disease genes interact with ATP7 to regulate copper distribution and availability in *Drosophila melanogaster* *Brooke Allen*, Alysia Vrailas-Mortimer Illinois State University

Copper is an essential element for enzymes that catalyze oxygen-dependent reactions. When an organism is exposed to either excess copper or deprived of copper, this micronutrient becomes detrimental. A mechanism used to control copper distribution and availability involves the ATPase transporter, ATP7. This X-linked transmembrane protein is responsible for delivering copper into the lumen of the cell by utilizing both endocytic and exocytic mechanisms. Mutations in ATP7 have been shown to cause Menkes disease and Wilson's disease, which both share the phenotype of neurodegeneration. These genetic disorders with ATP7 defects both lead to mechanisms of neurodegeneration that is likely shared with other, more common neurodegenerative diseases, such as Parkinson's disease. A screening of possible candidate genes that interact with ATP7 was conducted by inhibiting Parkinson's disease genes in a ATP7 loss of function or ATP7 overexpression background. We find that several of the Parkinson's disease genes showed a genetic interaction with ATP7, indicating that the mechanisms of neurodegeneration caused by ATP7 mutations may be conserved in Parkinson's disease. These interactions and their link to neurological disorders will further discussed.

782V Age-related neuroprotection by dietary restriction requires OXR1-mediated retromer function *Kenneth Wilson*[1], Sudipta Bar[1], Enrique Carrera[1,2], George Brownridge III[1], Jennifer Beck[1], Tyler Hilsabeck[1,3], Christopher Nelson[1], Geetanjali Chawla[4], Rachel Brem[1,3,5], Hugo Bellen[6], Lisa Ellerby[1], Pankaj Kapahi[1,3] 1) Buck Institute for Research on Aging, Novato, CA; 2) Dominican University of California, San Rafael, CA; 3) Leonard Davis School of Gerontology, University of Southern California, Los Angeles, CA; 4) Regional Centre for Biotechnology, Faridabad, Haryana, India; 5) Department of Plant and Microbial Biology, University of California, Berkeley, Berkeley, CA; 6) Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX

Dietary restriction (DR) is the most robust method to delay aging and the onset of neurodegeneration, though the mechanisms behind this phenomenon remain unclear. Further, it remains unknown which factors influence why different individuals will respond to dietary interventions to different degrees. We reared over 150 fully sequenced fly strains from the Drosophila Genetic Reference Panel under ad libitum feeding or diet-restricted conditions and measured lifespan as well as healthspan. Through genome-wide association study, we identified genetic variants associated with influencing these traits under each dietary condition. A variant in one gene, *mustard* (*mtd*, called *Oxidation resistance 1*, *OXR1*, in humans), significantly associated with DR-specific lifespan. We demonstrate that *mtd*/*OXR1* in neurons is necessary for DR-mediated lifespan extension and that neuronal overexpression of human *OXR1* is sufficient to extend lifespan upon DR in flies. Neuronal knockdown of *mtd* also inhibits dietary restriction-associated slowing of age-related visual decline, arguing for a specific role of *mtd*/*OXR1* in DR-mediated neuroprotection. We additionally identified that natural variants of *mtd* in the promoter region are associated with enhanced longevity upon DR and are regulated by the transcription factor Traffic jam (TJ). We further show that mtd is essential for stabilizing the retromer complex, which is necessary for trafficking transmembrane proteins for reuse. As a result of OXR1 deficiency, the retromer destabilizes and lysosomes become overused. Overexpression of retromer proteins or supplementation with chaperone compound R55 rescues the lifespan defects and neurodegeneration seen in mtd-deficient flies, and R55 is capable of rescuing lysosomal aggregation and OXR1-retromer co-localization in cells from humans with OXR1 deficiency. Thus, mtd/OXR1 enhances protein

recycling in response to DR through the retromer, improving neuronal health and lifespan.

783V A GWAS for late-life mortality in *Drosophila* identifies *Diabetes and obesity regulated* to regulate mortality and resilience. *Tyler Hilsabeck*[1,2], Kenneth Wilson[1], Jennifer Beck[1,3], Christopher Nelson[1], Rachel Brem[1,2,4], Pankaj Kapahi[1,2] 1) Buck Institute, Novato, CA; 2) University of Southern California, Los Angeles, CA; 3) University of California, San Francisco, CA; 4) University of California, Berkeley, CA

Variations in rate of aging in geneticaly heterogenious populations support the hypothesis that aging is at least partially genetically regulated. However, genetically identical individuals also vary in their time of death even when maintained in the same environment. We have observed in *D. melanogaster* that this variation is genotype dependent, as specific genotypes have characteristic survival curve shapes that are largely reproducible. We hypothesize that there is an underlying genetic component to this seemingly stochastic nature of lifespan curves. Typical aging studies reduce a strain's lifespan down to a population-level value, such as mean or max lifespan. While these metrics can represent the trends in a population, they are unable to encapsulate the variation in the aging of individuals from the same distinct population. Instead, we used two values that characterize the logistic fit of a strain's hazard ratio over its lifespan: the risk of initial mortality (α) and the rate of aging (β). To identify regulators of the rate of aging and stochasticity of lifespan, we performed a *Genome-Wide Association Study* (*GWAS*) of β for 160 different fly strains from the *D*GRP collection on two different diets late in life. This approach identified the candidate gene *Diabetes and Obesity-Regulated (DOR),* which has known roles in stress response, autophagy, and senescence, as having a role in the late-life mortality. *DOR* inhibition leads to a significant increase in late-life mortality that is preceded by a reduction in healthspan-related traits. Further, we've found that germline-specific inhibition is sufficient to shorten lifespan. We conclude that a decrease in expression of *DOR*, a conserved gene, compromises an organism's resilience, leading to a decline in healthspan and increased mortality, providing a potential target for bolstering the decline seen in human aging.

784V Inter-kingdom lipid transfer mediates D. melanogaster temperature-adaption *Claudia Espinoza*, Itay Budin University of California San Diego

Animals must undergo physiological modifications to adapt to yearly climate fluctuations. At low temperatures, cell membranes undergo a phase transition from liquid to gel-like state, lowering membrane fluidity and disrupting the function of essential membrane-associated machinery. In organisms that overwinter, a class of lipids in their membranes, polyunsaturated fatty acids (PUFAs) with 2-6 double bonds, can act to maintain membrane fluidity and function at low temperatures. However, most animals, including Drosophila, lack the desaturase enzymes responsible for the synthesis of PUFAs, and must rely solely on dietary sources to obtain this lipid class. This lipid exchange underlying the interplay between foraging behavior, food composition and cell membrane organization, which is crucial for organisms' seasonal adaptation, remains unexplored. Using Drosophila melanogaster and different yeast species as its dietary counterpart, we are investigating how PUFAs obtained through diet determine seasonal adaptation and what the mechanisms of PUFA detection and selection are. Analysis of D. melanogaster associated yeast collected at different times of the year allowed us to identify the increase in PUFA-producing species during the cold months. In the lab, we find that controlled dietary intake of yeast PUFAs dictates Drosophila activity at low temperatures. Lipidomics analysis shows how the presence of the key temperature-responsive PUFA linolenic acid is determined in specific lipid classes as a function of diet. We also find that temperature also determines both feeding and oviposition preferences, with flies favoring feeding from and laying eggs near PUFA-producing yeast at low temperatures. Using a combination of mass spectrometry, feeding assays, and yeast and Drosophila mutants, we are currently elucidating the yeast volatiles and Drosophila genetic pathways responsible for the detection and selection of dietary PUFAs at low temperature. This knowledge will help us understand the mechanisms by which lipid exchange influences seasonal adaptation in symbiotic organisms like yeast and Drosophila.

785V The impacts of sex and genetic background on the response of *Drosophila melanogaster* to essential and non-essential metal toxicity *Mitchell Slobodian*, Athena Wallis, Joshua Keeping, Allie Sutherland-Hutchings, Jesse Petahtegoose, Solomon Arthur, Danica Levesque, Thomas Merritt Laurentian University, Sudbury, Ontario

Drosophila melanogaster is an effective model of metal toxicity, but we currently have only a limited understanding of any sex or genetic background effects. Similarly, while essential metals are relatively well studied, many non-essential metals remain understudied despite their chemical similarities to essential metals and their relevance as environmental contaminants. Further, essential and non-essential metals are not independent. The two groups interact with multiple interconnected metal pathways in a complex web with many proteins and mechanisms yet to be elucidated. In the current study, we are quantifying the influence of sex and genetic background on the response to essential and non-essential metal toxicity in *D. melanogaster*. We are using copper (Cu) and nickel (Ni) as our essential and non-essential metals, both delivered through the fly food. The two are chemically similar metals and can bind to many of the same biological ligands. Our study also includes different homozygous fly lines from the *Drosophila* Genetic Reference Panel (DGRP) to examine background effects. To date, we have conducted multiple four-day exposure Lethal Concentration 50 (LC50) assays using a range of $CuSO_4$ and $NiSO_4$ concentrations. There are significant effects of sex and genetic

background for both metals. Background effects were particularly substantial, and interestingly, the same lines were sensitive or resistant to both metals. We also found similar sex effects and combined sex and line effects for the two metals. Currently, we are quantifying the amount the concentration of metals in the fly samples, not just the fly food using two different methods, one with the non-specific metal chelator 4-(2-pyridylazo)resorcinol (PAR) and the second with Inductively Couple Plasma Mass Spectrometry (ICP-MS). Future work will investigate the genetic and biochemical influences that cause the differences in response to metal toxicity and the effects of simultaneous exposure to both metals. Our results to date clearly demonstrate that sex and genetic background are major influences in the response to essential and non-essential metal toxicity. Understanding the biology of this system, and likely any other biological system, requires experiments with multiple genetic backgrounds and both sexes.

786V HIF-1α promotes hypoxia tolerance by restraining excess cytokine signaling *Kate Ding*[1,2,3], Danielle Polan[1,2,3], Byoungchun Lee[1,2,3], Tiffany Cheung[1], Elizabeth Barretto[1], Savraj Grewal[1,2,3] 1) University of Calgary, Calgary, AB; 2) Arnie Charbonneau Cancer Institute; 3) Department of Biochemistry and Molecular Biology

Our cells and organs need oxygen from the air we breathe in order to function normally. In conditions of low oxygen, also known as hypoxia, animals experience tissue damage and deregulation of metabolic homeostasis which are characteristic of diseases such as stroke and ischemia. Tumors are also associated with hypoxia, which is thought to trigger enhanced cell proliferation and metastasis. Furthermore, diseases that obstruct proper oxygen intake such as chronic lung disease and sleep apnea can result in intermittent, but chronic, whole-body hypoxia that can have long-term deleterious effects on health.

Although the air we breathe is made up of ~20% oxygen, our cells and tissues experience much lower levels than this, ranging from 1-10%. This aspect of normal physiology is often overlooked in cell culture experiments where the cells are typically maintained in 20% oxygen. Understanding how tissues and organs respond to low oxygen is therefore an important question in biology. *Drosophila* experience sustained conditions of hypoxia as they bury in rotting fruit they forage through during the larval stages. They have therefore evolved to tolerate hypoxia. Trachea deliver oxygen directly to tissues, hence by modulating environmental oxygen we can trigger hypoxia in cells and tissue. As such, *Drosophila* have emerged as an ideal in vivo model for studying how organisms tolerate hypoxia. Recent studies have revealed an adaptive role for Upd/JAK/STAT signaling in response to conditions of low oxygen that involve tissue-tissue crosstalk to promote differentiation of specific immune cells.

In our lab, we have demonstrated a role for cytokine signaling mechanisms - involving the fly interleukin-6 homolog *upd3*- which is important for whole-body hypoxia tolerance. We found that expression of *upd3* is strongly upregulated (>20-fold) in hypoxia and that *upd3* mutants show reduced survival in low oxygen. Interestingly, we also found that knockdown of HIF-1 alpha, the classic hypoxia-induced factor required for survival in low oxygen, lead to a further enhancement of *upd3* expression. Further, when we genetically mimicked this enhancement in *upd3* levels, we found animals also had reduced survival in low oxygen. 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 recent discovery that *upd3* is modulated in hypoxia and may be regulated in part by HIF-1α, this leaves open the question of what role *upd3* is playing and how it promotes hypoxia tolerance. Using the versatility of *Drosophila* genetics, we have begun to tease apart what tissues may be producing *upd3* and where the cytokines may be acting.

787V mTORC2 protects heart from HFD induced-damage through promoting mitochondrial fission Peiduo Liu Iowa State University

High fat diet (HFD)-associated lipotoxicity is one of the major causes of cardiovascular diseases. The mechanistic target of rapamycin (mTOR) pathway, especially mTOR complex 1 (mTORC1), has been previously implicated in HFD-induced heart dysfunction. In the present study, we find that unlike mTORC1, mTOR complex 2 (mTORC2) protects hearts from HFD-induced cardiomyopathy and mitochondrial dysfunction in Drosophila. We show that HFD feeding induces contractile dysfunction along with altered mitochondrial morphology and function. Upon HFD feeding, the mitochondria of cardiomyocytes exhibit fragmentation, decrease of membrane potential. Interestingly, short-term HFD feeding induces the activity of cardiac mTORC2 which could be a protective response. In line with this finding, the flies with cardiac-specific knockdown of rictor, the key subunit of mTORC2, show cardiac and mitochondrial dysfunction similar to what is observed in HFD-fed wild-type flies. Also the mitochondrial fission as an adaptive response is blocked by rictor knockdown. Conversely, cardiac-specific activation of mTORC2 by overexpressing rictor attenuates HFD-induced mitochondrial and cardiac dysfunction. Thus, our findings suggest that mTORC2 is a cardioprotective factor and may through promoting the mitochondrial fission.

788V Role of Wnt signaling in regulating lipid homeostasis in *Drosophila* *Rajitha Udakara Sampath Hemba-Waduge*[1], Xiahe Huang[2], Mengmeng Liu[1], Xiao Li[1,3], Yingchun Wang[2], Jun-yuan Ji[1] 1) Tulane University School of Medicine, New Orleans, LA; 2) Chinese Academy of Sciences, Beijing, China; 3) Princeton University, Princeton, NJ

Dysregulated Wnt signaling causes aberrant development and diseases such as cancer, but the role of Wnt signaling in regulating lipid metabolism remains poorly understood. We have reported that hyperactive Wnt signaling in *Drosophila* larvae reduces lipid accumulation in the fat body, which can be strongly rescued by feeding the larvae with proteasome inhibiting peptide boronic acids, such as Bortezomib. Interestingly, hyperactive Wnt signaling in adipocytes increases the levels of different types of free fatty acids but reduces the levels of a variety of triglycerides. To elucidate the mechanisms of how Wnt signaling regulates lipid homeostasis in adipocytes, we have performed transcriptomic and quantitative proteomic analyses using larvae with hyperactive Wnt signaling with or without Bortezomib treatment. These analyses led us to identify genes involved in regulation of lipid mobilization, such as *PLIN1* (*Perilipin1*), *PLIN2*, and genes encoding factors that regulate fatty acid beta-oxidation. Both *PLIN1* and *PLIN2* are significantly reduced by hyperactive Wnt signaling, but these effects are strongly reversed by Bortezomib treatment. Our genetic analyses suggest that adipocyte defects caused by hyperactive Wnt signaling can be strongly enhanced by depleting *PLIN1*, but rescued by ectopic expression of *PLIN1*, *PLIN2*, or both. Taken together, these observations suggest that Wnt signaling may regulate lipid homeostasis by promoting lipid mobilization through regulating the expression of *PLIN1* and *PLIN2* in larval fat body.

789V Identification of direct targets of Bortezomib in *Drosophila* using a chemical proteomics approach Mengmeng Liu Tulane University

Identification of direct targets of Bortezomib in *Drosophila* using a chemical proteomics approach

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Wnt signaling plays critical roles in regulating diverse biological processes, and deregulated Wnt signaling causes aberrant development and diseases such as cancer. We have previously reported that hyperactive Wnt signaling disrupts lipid homeostasis in *Drosophila* larvae, which can be strongly rescued by feeding the larvae with Bortezomib (BTZ) in an a-Catenin-dependent manner. BTZ is the first proteasome inhibitor approved by the Food and Drug Administration to treat patients suffered from multiple myeloma and mantle cell lymphoma. We have found that BTZ and two additional peptide boronic acids (PBAs), Delanzomib and Ixazomib, can rescue adipocyte defects caused by elevated Wnt signaling, but three other proteasome inhibitors that have different chemical structures (Carfilzomib, Marizomib, and Oprozomib) cannot rescue the phenotypes. These observations indicate that PBAs may have additional targets besides proteasome. To elucidate how PBAs regulates lipid homeostasis and Wnt signaling, we synthesized a photoactivatable biotin-tagged BTZ, which displays similar effects on rescuing adipocyte defects and inhibiting Wnt activity to BTZ. Using this biotin-tagged BTZ, we performed a pull-down assay using streptavidin beads followed by mass spectrometry analysis to identify proteins bind to BTZ. We have identified 126 candidate targets of BTZ, including majority subunits of the proteasome complex as expected. Interestingly, we also identified some peptidases and proteinases. We are performing genetic and cell biological analyses to determine whether these peptidases and proteinases play any roles in regulating a-Catenin stability and Wnt signaling in *Drosophila*. This study may advance our understanding of the molecular mechanism of how BTZ regulates a-Catenin stability and Wnt signaling *in vivo*.

790V Odor mediated control of blood-progenitor redox homeostasis in *Drosophila* *Manisha Goyal*[1,2], Ajay Tomar[1,2], Sukanya Madhwal[1], Tina Mukherjee[1] 1) Institute For Stem Cell Science and Regenerative Medicine (inStem); 2) The University of Trans-Disciplinary Health Sciences & Technology (TDU)

The importance of reactive oxygen species (ROS) in myeloid cell development and function is well established. However, any aberrant generation of ROS alters hematopoiesis. Thus, maintaining homeostatic levels of ROS is very crucial for progenitor development. While the autonomous mechanism of ROS homeostasis is explored but any physiological level interpretation that regulates this ROS homeostasis remains largely unclear. Here, we show a sensory regulation of redox homeostasis in blood-progenitor cells which is mediated by olfaction. We find that this long-range communication, to facilitate ROS homeostasis is central to the development of blood progenitor cells and successful immune response. This work shows yet another implication of environmental control of myeloid physiology at the axis of ROS homeostasis by odors and GABA. GABA metabolism in blood-progenitor cells regulate TCA cycle activity and antioxidants synthesis to control ROS balance in the lymph gland. We have identified the metabolic requirement of odor sensing and GABA in regulating redox homeostasis during *Drosophila* myeloid progenitor development, the relevance of which may be broadly conserved.

791V Effects of Ambient Temperature on Body Fat *Jin Seo*, Rachael Winfrey, Alexander Jochmans, Eunji Yoon Rogers State University

Temperature profoundly affects all living organisms on development, growth, longevity, and metabolism. Ambient temperature significantly alters lipid/ carbohydrate storages in fruit flies; conversely, food intake alters flies' preferred

temperature. The natural thermal range of fruit flies is between 13 and 33 °C. When adult flies are incubated at a higher natural thermal temperature, body fat contents are strikingly reduced. Remarkably, fat loss does not appear to be due to decreased food intake and/or increased metabolic rate suggesting that temperature-mediated fat storage is regulated through active mechanisms rather than passive processes. The rates of biochemical reactions show a bell curve pattern in which the reaction rates increase up until the optimum temperature (T_{opt}) and drop sharply beyond the T_{opt}. To better understand mechanisms of temperature-mediated energy storage and identify thermosensing molecules, we have investigated enzyme activities in multiple signaling pathways, which are linked to lipid/carbohydrate metabolism, at suboptimum temperatures.

792V Lifestyles and metabolism of *Drosophila lutzii*, a floridosa group of species, and sympatric *D. simulans*, a generalist specie *Juan Manuel Murillo-Maldonado*, Juan R. Riesgo-Escovar Universidad Nacional Autónoma de México

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 characterize *Drosophila lutzii*, a Neotropical Phloridosa group of species of *Drosophila*. As its group of species implies, they are not saprophytic, but rather feed on flowers. We made a comparative study between *D. lutzii,* a specialist, and sympatric *D. simulans*, a generalist. We analyzed metabolic and behavior parameters. *Drosophila simulans* is a saprophytic generalist, with feeding based on rotting plants and fruits, while *Drosophila 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, as compared to sympatric freshly caught *D. simulans.* Consistent with a restricted diet and specialist lifestyle, *D. lutzii* flies are less capable of surviving in culture in diets that differ in the amounts of carbohydrates, and 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 that varied 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 in 30 minutes periods. 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.

793V Genetic analysis of *Juvenile hormone epoxide hydrolases* in *Drosophila* *Felipe Rogalski*, Toshiro Aigaki Tokyo Metropolitan University, Tokyo, Japan

Juvenile hormone epoxide hydrolases (JHEHs) are enzymes that inactivate juvenile hormone (JH) in insects. The inactivation occurs through the hydrolysis of the epoxide group within the hormone into diol. JH's main function is to ensure larval growth while preventing metamorphosis, but it also affects adult processes, such as egg production, fertility, and behavior. The genome of the fruit fly, *Drosophila melanogaster*, encodes three JHEHs: *Jheh1*, *Jheh2*, and *Jheh3*. It is not known whether those genes play a major role in JH related phenotypes. *Jheh1* and *Jheh2* have been repeatedly identified as oxidative stress response genes since they are induced by several xenobiotics, like paraquat and caffeine. Recently, JHEH1 and JHEH2 have been shown to interact with a lipid droplet associated protein, suggesting a role in lipid metabolism. *Jheh3*'s function is completely unknow. To understand the roles of JHEHs in the fruit fly, we investigated the impact of genetic disruption of *Jhehs*. We focused on *Jheh1* and *Jheh2* since they might be involved in resistance against stresses. Using the CRISPR/Cas-9 system, we have generated a mutant deleted for *Jheh1* and *Jheh2*. The mutant flies were viable, fertile, showed a normal lifespan and there was no difference in the resistance against paraquat or caffeine between wild-type and Jheh1-Jheh2 KO flies. *Jheh1-Jheh2 KO* flies showed decreased body weight and reduced amount of triacylglycerol (TAG). Conversely, overexpression of *Jheh1* using the Gal4-UAS system increased TAG storage and body weight. JH is known to control developmental speed. *Jheh1-Jheh2 KO* flies had a slight delay in development, however, the expression level of *krüppel-homolog 1* (*kr-h1*), a JH-responsive gene, was not different between wild-type and mutant flies, suggesting that JH signaling was not involved in the mutant phenotype. To obtain insights into the metabolic processes of *Jheh* mutant flies, we performed metabolomic analysis using liquid chromatography-mass spectrometry (LC-MS). All glycolytic metabolites were decreased in *Jheh1-Jheh2 KO* flies, whereas TCA cycle metabolites remain normal. These results indicate that glycolytic activity is low in the mutants. In conclusion, our research shows that *Jheh1* and *Jheh2* are involved in the regulation of growth rate, glycolysis, and lipid metabolism, demonstrating that they are essential for energy homeostasis in *Drosophila*.

794V Developmental Exposure to the PFAS molecule, PFOA, alters Lipid Homeostasis in *Drosophila Melanogaster* *Eric Kilbourn*, Jason Tennessen Indiana University Bloomington

Perfluoroalkyl substances (PFAS) are man-made environmental contaminants that are now found on all seven continents. This family of compounds has been linked to heath issues such as liver cancers, decreased efficiency in vaccines, and obesity. The most prevalent of these compounds, perfluorooctanoic acid (PFOA), has a predicted environmental half life

of over 1000 years, meaning dozens of future generations be exposed to this molecule and the broad range of related PFAS chemicals. Despite the serious health concerns with PFAS, and PFOA in particular, relatively few mechanistic studies have examined the effects of PFAS *in vivo*. Here we address this deficiency by examining the effects of PFOA on *Drosophila* metabolism. Our preliminary results reveal that flies readily absorb PFOA from dietary sources and accumulate this molecule to detectable levels. Moreover, our preliminary results reveal that developmental exposure of PFOA induces abnormal lipid droplet accumulation in the larval fat body. We are now examining the effects of PFOA exposure on both triglyceride metabolism as well as the metabolism of other lipid species. Overall, our data shows that that *D. melanogaster* is a promising model for the investigating the effects of PFAS exposure on human health.

795V Exploring pathophysiology in long-lived fly populations reared on two diets *Utsav Nyachhyon*, Thomas Rundell, Laura Musselman Binghamton University

Overnutrition in Drosophila melanogaster and in humans causes pathophysiology including increased infection susceptibility, heart failure, insulin resistance, hyperglycemia, and reduced lifespan that are associated with obesity and other markers of metabolic disease. We used Drosophila as a model organism to identify genes that contribute to fitness and longevity during overnutrition. A large outbred population was selected for the ability of flies to survive chronic overnutrition conditions of high-sugar feeding. Every generation, half of the population that survived were bred to produce the next generation. Over time, the lifespan of high-calorie-adapted flies was extended, compared to those fed control diets. We hypothesize that improved heart function and/or immunity of the high-calorie-adapted flies might contribute to their increased survival. To test this hypothesis, these adapted flies are being subjected to cardiac stress and infection studies. In cardiac pacing, the flies are electrically stimulated to quantify the rate of heart failure and recovery. In infection studies, the flies are inoculated with Pseudomonas aeruginosa to quantify their infection susceptibility. Heart and immune function are being examined in control and adapted populations to search for mechanisms by which increases in the lifespan and healthspan may have occurred during selection. Significant changes in pathophysiology of adapted populations in comparison to control populations will be explored further by the ID of genes associated with these changes. Future studies will test the role of these genes which may be novel, conserved regulators of fitness during overnutrition in animals.

796V Optimisation of macro- to micronutrient balance for larval growth on a holidic diet *Sebastian Sorge*, Panayotis Pachnis, Vanessa Tixier, Alex Gould The Francis Crick Institute, London

The contributions of individual macro- and micronutrients to *Drosophila* phenotypes can be untangled using holidic diets. Currently available holidic diets are useful for studying adult nutrition and lifespan but larval growth and/or development are substantially slower than on standard yeast-based diets. We previously published a chemically-defined diet (CDD) related to other published holidic diets and with a similarly poor larval performance. We now describe semi-systematic improvements to our CDD that substantially speed up larval growth and development, close to values obtained with a yeast-based diet. Our results confirm that amino-acid composition influences developmental speed but we also find that the precise macro- to micro-nutrient balance is of similar importance. Several non-essential micronutrients contribute to larval developmental progression to surprising degrees and even small changes in the nutrient balance can substantially perturb development. Together, these CDD studies help to define a narrow window of macro-to-micronutrient balance that is optimal for larval development. As a proof-of-principle, we apply the optimised CDD to larval stable isotope tracing experiments, determining the sugar carbon contribution to hemolymph polar metabolites and to de-novo synthesized fatty acids.

797V The conquest of a new habitat: A study of the nutritional and sensory adaptations of the *D. suzukii* larvae. *Diego Galagovsky*, Ana Depetris-Chauvin, Markus Knaden, Bill Hansson Max Planck Institute for Chemical Ecology

Animals need to feed to incorporate nutrients –protein, carbohydrates, and lipids, to use as source of energy and as building blocks to maintain their bodies. However, feeding substrates contain nutrients in different proportions; therefore, depending on the specific needs of an organism, food sources will have varying nutritional quality. To assess their needs and feed accordingly, animals have both mechanisms to evaluate their internal needs, and mechanisms to sense for chemical cues in foods that hint at their nutrient content. However, for some organisms, it is not their decision where to feed, but that of their parents.
In the life cycle of Drosophila flies, we can distinguish two main feeding stages–the larva and the adult, with different objectives and nutritional needs. The larva feeds mainly to grow and accumulate resources for metamorphosis while the adult needs nutrients to survive and achieve reproduction. Adult *D. melanogaster* flies prefer to feed and lay eggs on protein-rich overripe fruits. These make a good feeding substrate for the larvae. Conversely, while *D. suzukii* adult flies also feed on overripe fruit, they are physically and behaviorally adapted to lay eggs on ripening fruits, which are harder and have a high sugar-protein ratio. *D. suzukii* larvae have to face the harsh nutritional landscape that has resulted from this novel adult behavior. How do they do it? Are the larvae physically adapted to this medium? How have their nutritional needs, their behavior and their physiology adapted to the shift in adult ecology?

I am addressing these questions at different levels; the nutritional composition of host fruits, the larval behavior, and the larval physiology.
Our results indicate that the environment in which *D. suzukii* larvae develop, the ripening fruit picked by their mother, is not a temporally homogeneous environment. It starts out as a sugar-rich, protein-poor environment, in which *D. melanogaster* larvae cannot survive. As the *D. suzukii* larvae process it, the protein content of the fruit increases. Their presence and activity also stimulate the fermentation of the fruit. By the time the larvae reach the late stages, the feeding substrate has become rich in proteins, enough to sustain *D. melanogaster* development. We have also observed that, compared to *D. melanogaster,* the 1st instar larvae of *D. suzukii* have massive mouth hooks, which aid them in processing the hard ripening fruit. Do *D. suzuki* larvae have special behaviors associated to the use of their physical adaptations? How does their physiology deal with the composition of the medium before they convert it? We are currently exploring the behavioral and physiological characteristics of the early larvae that allow them to develop in the ripening fruit.

798V Oxidative stress resistance in insulin-signaling impaired male and female *Drosophila melanogaster* *Jessica Alvarez*, Juan Rafael Riesgo-Escovar UNAM, Queretaro, Mx

Diabetes encompasses a group of metabolic disorders characterized by disglycemia, caused by insufficient insulin production or its inefficient signaling, and entails detrimental consequences for the patient's quality of life. *Drosophila melanogaster* has an evolutionarily conserved insulin signaling pathway. We set to understand how an organismal deficiency in insulin signaling in adult male and female flies affects different aspects of the fly›s life, particularly the response to oxidative stress.
We exposed *InR* (the insulin receptor fly homolog) and *dS6K* (the ribosomal protein S6 kinase beta-1 fly homolog, under the control of TORC1, also regulated by insulin signaling) heteroallelic mutants, as well as wildtype controls with the same genetic background, to different prooxidant compounds. We found that females have higher lipid and carbohydrate accumulation and show better resistance to oxidative stress (H_2O_2) than males. A heterozygous mutation in *Keap1* (a negative regulator of *CncC*, a master regulator of the antioxidant response) only rescued the phenotype of mutant *InR* males. We conclude that the type of oxidant fed to the fly and the genetic manipulation of the antioxidant response affected differentially the survival of males and females.

799V The Role of Copper in Parkinson's Disease *Jessica Burkhart*, Alysia Vrailas-Mortimer Illinois State University

Parkinson's Disease (PD) is a neurodegenerative disease caused by the death of dopaminergic neurons in the substantia nigra region of the brain. PD is characterized by the presence of dysfunctional mitochondria and increased levels of oxidative stress. Though a handful of genes, such as parkin and PINK1, have been identified in familial forms of PD, most cases are sporadic. Therefore, it is thought that environmental factors may act on genetic risk factors to promote disease onset. Therefore, we are exploring the relationship between copper toxicity, which has been linked to other neurological disorders, and parkin and PINK1. We are testing the effect of environmental exposure to copper as well as altering copper levels genetically by manipulating the copper transporter ATP7, which is mutated in the neurodegenerative disorder, Menkes disease. Preliminary findings have shown that increased extracellular copper, from over-expression of ATP7, in conjunction with knockdown of parkin and PINK1 exasperate Parkinson's symptoms.

800V Determining the mechanism of anesthetic-induced neurotoxicity in a Drosophila model of mitochondrial disease *Zachariah Olufs*, David Wassarman, Misha Perouansky University of Wisconsin - Madison, Madison, WI

General anesthetics are broadly used and are safe in healthy individuals. However, adverse sequelae collectively referred to as anesthetic-induced neurotoxicity (AiN) have been reported in pre-clinical animal models and clinical studies of mitochondrial disease. Leigh Syndrome (LS) is the most common pediatric manifestation of mitochondrial disease. Over 75 distinct genes underlie LS, including mutations in Complex I of the mitochondrial electron transport chain. To investigate the mechanism of AiN, we have characterized flies carrying a mutation in *ND23*, which encodes a core subunit of Complex I and is orthologous to mammalian *NDUFS8.* We previously found that *ND23* mutants are behaviorally sensitive to two volatile general anesthetics (VGAs), isoflurane (Iso) and sevoflurane (Sevo). Furthermore, Iso, but not Sevo, causes mortality within 24 hours of exposure, the extent of which is dependent on oxygen concentration ($[O_2]$) during exposure; low $[O_2]$ (hypoxia) reduces mortality and high $[O_2]$ (hyperoxia) increases mortality. To identify genes involved in the differential response of *ND23* mutants to different anesthetics and to different oxygen concentrations, we performed RNA-seq analysis of fly heads. We found that Iso and Sevo differentially affected expression of chaperone and antioxidant genes, but high and low $[O_2]$ did not appreciably influence gene expression. To test whether differential expression of chaperones or antioxidant genes is responsible for the difference in mortality between Iso- and Sevo-exposed flies, we used orthologous approaches to induce the transcriptional responses prior to anesthesia exposure, since exposure to a non-injurious stress might initiate protective mechanisms that would result in a state of tolerance to the subsequence injurious stress. We found that pre-exposure to Sevo or heat shock did not reduce mortality from a subsequent exposure to Iso. These data argue against a role for transcriptional activation of chaperone and antioxidant genes in the mortality mechanism. We are currently testing other non-injurious stressors as well as using loss-of-function

genetic approaches to test whether the transcriptional response or excess oxygen are necessary for Iso-induced mortality in the context of the *ND23* mutant.

801V Role of peroxisome in mitochondrial dynamics during aging in *Drosophila melanogaster* *Ankur Kumar*, Pham Vo, Peiduo Liu, Hua Bai 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 and function 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 peroxisomal plasmalogen synthesis, such as GNPAT (Glyceronephosphate O-acyltransferase) resulted in enlarged mitochondria. Alterations in other peroxisomal genes also have changed the mitochondrial morphology. Thus, our future goal is to understand how peroxisomes contribute to age-related alterations of mitochondrial dynamics and functions.

802V Transcriptional regulator of DR responsive genes extends lifespan and regulate Tau pathology in *Drosophila* *Sudipta Bar*, Patrick Wai-Lun Li, Tyler Hilsabeck, Guiping Du, Simon Melov, Pankaj Kapahi Buck Institute for research on aging

Dietary restriction (DR) is a robust intervention that slows aging in multiple species and staves off age-related diseases, including Alzheimer's disease and related dementias (ADRDs). Despite identifying some of the genetic risk factors for Alzheimer's disease (AD), therapeutics to treat ADRDs have been unsuccessful. However, the underlying mechanisms by which DR protects against AD are unknown. Identifying the mechanism behind the DR-mediated neuroprotection and lifespan extension can lead us to new therapeutic targets for AD. Using Translating Ribosome affinity purification (TRAP) method we have identified tissue-specific gene expression profiles of *Drosophila* in *Ad libitum* (AL) and DR conditions. We have identified a relatively large proportion of the unique DR-responsive genes to be altered in neurons. Utilizing bioinformatics approaches and publicly available chromatin immunoprecipitation sequencing (ChIP-seq) data from the ENCODE consortium we identified transcription factors (TFs) that are significantly enriched at promoter regions of genes that are upregulated or downregulated in fly brains on DR, compared to flies reared on AL diet. Screening of those TFs in lifespan extension revealed downregulation of Jarid2 extends lifespan and rescues tau pathology in a fly model of AD. We determined there are 359 downstream target genes of Jarid2 among which homolog of 16 genes have been reported to influence AD risk. Gene ontology (GO) enrichment analysis of the Jarid2 downstream targets identified enrichment of several biological processes including system development, nervous system development, neurogenesis, axon development, etc. We identified the downregulation of a neurogenesis factor NetB, a downstream target of Jarid2, which abrogates the DR effect. Hence we are working to understand the role of Jarid2 and NetB in neuroprotection to establish as potential targets for AD.

803V The bestrophin-1 chloride channel is required in the Malpighian tubules and hindgut for osmoregulation in response to high salt diet John Pleinis, Sima Jonusaite, Katherine Beebe, Daryl Morrison, Forest Streeter, Jacob Hudac, Austin Goodwin, Ashlee Roberts, Jeffrey Schellinger, Adrian Rothenfluh, *Aylin Rodan* University of Utah

The mechanisms allowing *Drosophila melanogaster* to tolerate a high-salt diet are poorly understood. Here, we study flies carrying a mutation in the chloride channel, *Bestrophin-1* (*Best1*). Flies with a loss-of-function mutation in *Best1, Best1^{c04106}*, have increased lethality when fed a high-salt (0.3 M NaCl) diet. High salt lethality is rescued in mutant flies carrying a genomic rescue construct, or with expression of wild-type *Best1* in the Malpighian tubules and the rectal pads of the hindgut, the iono- and osmoregulatory epithelia of insects. Malpighian tubules from *Best1* mutants have impaired fluid secretion and ion flux. This is likely due to impaired ability to recycle chloride entering through the sodium-potassium-2-chloride cotransporter (NKCC), as there is no effect of *Best1* knockdown in flies carrying a loss-of-function mutation in the NKCC-encoding *Ncc69. Best1* mutants have lower hemolymph osmolality when fed a normal diet, and fail to increase osmolality as much as controls when fed a high-salt diet. This phenotype is also rescued by the genomic rescue construct, as well as by expression of *Best1* in the Malpighian tubule and rectal pads. High salt diet leads to dehydration in control flies, and this is further exacerbated in the *Best1* mutants, likely due to greater osmotic loss of water from the hemolymph into the high osmolar gut lumen, as suggested by increased excretion and excreta osmolality in *Best1* mutants. Analysis using specific GAL4 lines indicates that coordinated action of the Malpighian tubules and the hindgut is required to modulate excretion and dehydration. Metabolomic analysis indicates that urea is the most upregulated solute in control flies, whereas *Best1* mutants fail to upregulate urea. Accordingly, feeding *Best1* mutants

urea partially rescues high salt lethality, while having no effect on survival in control flies. *Best1* mutants have seizures, as occurs in patients with tonicity disorders. *Best1* expression in the Malpighian tubules and rectal pads rescues the seizure phenotype, indicating that it is due to the osmoregulatory defects, whereas *Best1* knockdown in neurons or glia does not result in seizures. Finally, *Best1* mutants sleep less, likely due to searching for water, as water supplementation restores sleep. Thus, *Best1* plays an essential role in the Malpighian tubules and hindgut to allow *Drosophila* to cope with salt stress.

804V The epicuticular lipid barrier is highly dynamic across the life course in *Drosophila* *Lena Lampe*[1], Clare L Newell[1], Ian Gilmore[2], Alex P Gould[1] 1) Francis Crick Institute; 2) National Physical Laboratory

An epicutaneous lipid barrier is found in all terrestrial organisms and protects them from dehydration and other potentially harmful stresses in the environment. The high surface area to volume ratio of insects means that an effective epicuticular lipid barrier to dessication is particularly important. The composition of the barrier lipid blend is highly complex and, although hydrocarbons have been extensively analysed, other cuticular lipid species are less well investigated. Here we use a new surface-specific technique for mass spectrometry, OrbiSIMS, to characterize the cuticular lipid repertoire of *Drosophila melanogaster* during ageing. We identify epicuticular ceramides, wax esters, phospholipids and free fatty acids and show that they have specific temporal profiles that change substantially during ageing. Using stable isotope tracing, we measure the turnover rates of epicuticular lipids and show that larval nutritional resources contribute greatly to some but not all molecular species. This study shows how larval nutrition contributes to the adult epicuticular lipid blend and the quality of the dessication barrier.

805V Endogenous degradation of hormones by two distinct classes of enzymes uniquely impact coordinated animal growth and development *Rebecca Spokony*[1], Lacy Barton[2], Ruth Lehmann[3] 1) Baruch College, CUNY, New York, NY; 2) Skirball Institute at NYU School of Medicine, New York, NY; 3) The Whitehead Institute at MIT, Boston, MA

Juvenile hormones (JHs) are central coordinators of insect development. These potent small molecules regulate developmental progression between different tissues as well as respond to changes in external conditions. JH titers are dynamic throughout development and the bioavailability of JH is presumed to be tightly controlled by dynamic expression of enzymes responsible for its synthesis and degradation. Two major classes of JH degradation enzymes are Juvenile hormone esterase (Jhe) and Juvenile hormone epoxide hydrolase (Jheh). Due to the importance of JH regulation, insecticides have been designed to block their functions to inhibit the maturation of insect pests and disease vectors.

The endogenous roles of these two distinct classes of JH degradation enzymes in Drosophila are not understood. This knowledge gap may arise from the fact that the *D. melanogaster* genome encodes two Jhe genes and three Jheh genes. Using CRISPR, we generated *Jhe, JheDup* null double mutant animals and *jheh1,2,3* null mutant animals to understand the functions of these two classes of enzymes. We find that while both *jhe,jheDup* and *jheh1,2,3* larvae exhibit delayed pupariation, the delayed maturation in *jheh1,2,3* triple mutant animals is much more dramatic, pupariating three days later than matched wildtype larvae. Interestingly, *jhe,jheDup* pupae and adults are significantly larger than matched wildtype animals, while *jheh1,2,3* animals are smaller. These findings suggest that while all these enzymes can degrade JHs, each class has tissue-specific functions that impact coordinated development and growth. We are currently investigating this exciting postulate, as well as compensatory feedback mechanisms across JH enzymatic classes. Our findings are consistent with global knockdown studies in *Bombyx mori*[1], *Leptinotarsa decemlineata*[2], and *Nilaparvata lugens*[3]. Thus, the novel genetic tools we generated in Drosophila will allow us to harness the powerful of this model organism to understand the spatiotemporal and tissue-specific functions of these important enzymes.

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806V Impacts of Intestinal Occluding Junction Modulation on Non-Cell Autonomous Hallmarks of Aging *Anna Salazar*, Cody Sessions, Kayla Pauley, Sierra Boyce Christopher Newport University, Newport News, Virginia

Intestinal barrier function is tightly linked to longevity in *Drosophila melanogaster* and other organisms. We have previously shown that altered expression of occluding junctions in the guts of fruit flies can lead to various hallmarks of aging, including modulation of intestinal homeostasis, variations in microbial dynamics, changes in immune activity, and alterations in lifespan. Loss of a specific occluding junction, Snakeskin (Ssk), leads to rapid and reversible intestinal

barrier dysfunction, altered gut morphology, dysbiosis, and a dramatically reduced lifespan. Remarkably, restoration of Ssk expression in flies showing intestinal barrier dysfunction rescues each of these phenotypes previously linked to aging. Intestinal up-regulation of Ssk protects against microbial translocation following oral infection with pathogenic bacteria. Furthermore, intestinal up-regulation of Ssk improves intestinal barrier function during aging, limits dysbiosis, and extends lifespan. Additionally, perturbing barrier function in the gut has non-cell-autonomous impacts, including alterations in the brain and muscle. Moreover, 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. These findings indicate that intestinal occluding junctions may represent prolongevity targets in mammals, in addition to their possible roles in intestinal dysfunction, aging, and disease. They also attempt to gain a better understanding of the impact of intestinal dysfunction on cells outside of the gut.

807V Single-nucleus RNA-seq of Drosophila Thorax Post Exercise Treatment: Pilot Study *Bre Minniefield*, Nicole Riddle University of Alabama at Birmingham

Physical activity in any form can significantly decrease the incidence of various diseases and benefit overall health. However, we are unable to recommend optimized exercise treatments for a specific individual's health needs. Despite significant progress, we lack a thorough understanding of the biological pathways that are activated or impacted by exercise. For example, little is known about how individual tissues are affected by exercise treatments and which cell- and tissue-levels change in response to exercise. Additionally, little is known about the pathway(s) that mediate short- and long-term exercise responses. Identifying molecular mediators of exercise response has the potential to provide trackable factors to better predict the outcomes of exercise for an individual, thus, allowing for optimized and personalized exercise treatments.

Here, we use a Drosophila exercise model to gain an understanding of the cell- and tissue-level changes precipitated by a short-term exercise treatment. Rotational stimulation by rotating the fly enclosures has been shown to increase animal activity in Drosophila, modeling exercise. Depending on genotype, animals treated with rotational stimulation show increases in activity up to 10-fold, and this increased activity alters gene expression, metabolite levels, and body condition. Using our established protocols with the TreadWheel exercise system, here, we carry out a single-nucleus transcriptome study focused on muscle to compare exercise-treated and control animals. Specifically, we use a wild-derived strain, DGRP line 304, which in previous studies showed a significant change in activity level with rotation and a change in weight as response to exercise in both sexes. Exercise-treated animals experienced five consecutive days of rotational stimulation for 2hrs, while control animals do not. Post exercise, thorax dissections were performed, a body part which consists of ~95% muscle. Using 10xGenomics technology, we collect single-nucleus transcriptome profiles from this tissue. We use these profiles to identify expression patterns, key cell types, and molecular pathways that are 1) differing between exercised and controlled flies; and 2) significantly affected by exercise. The analysis is in progress, and results will be reported. Discovering the key elements of exercise response will provide the foundational data to develop effective, personalized exercise plans, including alternatives for individuals unable to exercise.

808V dFNDC5 Regulates Exercise Performance and Adaptations in Drosophila *Tyler Cobb*[1], Jun Hee Lee[2], Myungjin Kim[2], Robert Wessells[1] 1) Wayne State University; 2) University of Michigan

Endurance exercise has profound benefits on health and mobility, but the mechanisms underlying these benefits are not fully understood. Identification of targets that mediate the benefits of exercise can be useful for the development of therapies to improve health in populations that cannot exercise or display exercise intolerance. We have developed a model to exercise train Drosophila and have shown that many adaptations in mobility, metabolism, and gene expression are conserved. Using this model, we have identified dFNDC5 as a key mediator of endurance exercise performance and adaptations. dFNDC5 is a homolog of mammalian FNDC5, which encodes a transmembrane protein expressed in muscle that is cleaved during exercise to give rise to a peptide called Irisin that regulates systemic adaptations in mammals. However, its role in exercise performance and endurance adaptation has not been studied in depth. We show that dFNDC5 mutants have impaired baseline endurance and do not adapt to chronic exercise training. Tissue-specific experiments reveal that dFNDC5 is required in muscle for normal baseline running endurance and for the ability to adapt to exercise. We also show muscle-specific dFNDC5 overexpression extends baseline endurance and is sufficient to promote mobility adaptations in unexercised flies. These findings suggest a conserved role of dFNDC5 in the exercise response.

809A Serotonin autoreceptors regulate *Drosophila* serotonergic axon morphology *in vitro* *Delaney Long*, Dayle Matheny, Luke Brewer, Douglas Roossien Ball State University

Serotonergic neurons produce extensively branched axons that fill most of the central nervous system, where they modulate behaviors such as mood, sleep, appetite, locomotion, and cognition. Proper behavioral output therefore depends on the precise outgrowth and targeting of serotonergic axons during development. To assist in this process, serotonergic neurons utilize serotonin as a trophic signaling molecule prior to it assuming its canonical role as a

neurotransmitter. This process, termed autoregulation, plays a negative role in *Drosophila* by limiting axon outgrowth, branching, and varicosity development. Yet the underlying mechanism of serotonin autoregulation remains unknown. In non-serotonergic neurons, activation of serotonin receptors causes downstream signaling events that are linked with F-actin depolymerization, growth cone collapse, and reduced neurite outgrowth. We therefore hypothesized that serotonin autoreceptors initiate autoregulation in serotonergic neurons. To test this, we adapted a primary neuron culture system in which *Drosophila* serotonergic neurons could be grown and unambiguously identified using Gal4-dependent expression of the fluorescent protein tdTomato. Next, we initiated autoregulation by applying exogenous serotonin to the culture. We found reduced axon outgrowth and branching, suggesting serotonergic neurons in culture respond similarly as previously reported *in vivo*. Lastly, we used pharmacological activation of serotonin autoreceptors to test their role in autoregulation. Axons treated with receptor agonists showed reduced outgrowth and branching, thereby mimicking the effects of serotonin application. This suggests that serotonin initiates autoregulation through activation of autoreceptors.

810B Investigating mechanisms of Frazzled/Dcc signaling in axon guidance *Sarah Gagnon*, Yixin Zang, Greg J. Bashaw
University of Pennsylvania, Philadelphia, PA, USA

Precise wiring of neural circuits is essential for the execution of vital functions, from breathing to complex motor movements. This connectivity is established during development as the axons of newborn neurons extend through the embryonic environment, sensing both attractive and repulsive cues via guidance receptors. In the central nervous system (CNS), commissural neurons send their axons across the midline to connect to contralateral targets. This is essential for left-right coordination, yet the underlying mechanisms are not fully understood. In flies, the guidance receptor Frazzled (Fra), like its human ortholog Deleted in Colorectal Cancer (Dcc), promotes axon growth toward the midline. Despite its critical role in axon guidance, our understanding of Fra/Dcc signaling remains incomplete. We performed an affinity purification-mass spectrometry screen in fly embryonic neurons to identify novel Fra interactors. We selected three candidates, the Toll-like receptor family member Toll-7, the iRhom pseudoprotease family member rhomboid-5 (rho-5), and the exportin embargoed (emb), all expressed in the embryonic CNS, for further investigation. Toll-7 regulates neuronal connectivity in the fly olfactory system, but its involvement in Fra signaling has not been investigated. Human iRhoms promote ADAM17 metalloprotease activity, which was shown by our lab to cleave and positively regulate the functions of both Fra and Dcc, making rho-5 a promising candidate regulator of Fra signaling. Finally, as nuclear export antagonizes the transcriptional activity of Fra, emb may also modulate Fra signaling. Our goal is to characterize the roles of Toll-7, rho-5, and emb in Fra and Dcc signaling. We show that Toll-7 and emb biochemically interact with Fra in S2R+ cells. Importantly, we find that all three candidates modulate midline crossing: Toll-7 and rho-5 promote crossing, while emb antagonizes this process. Given their physical association with Fra, it is likely that the candidates regulate midline crossing via the Fra pathway. Future experiments will determine whether Toll-7, rho-5 and emb function in the Fra pathway and if these genes play conserved roles in vertebrate Dcc signaling. Our research will shed light on uncharacterized mechanisms of Fra/Dcc signaling and axon guidance in *Drosophila* and vertebrates. These findings will provide insight into the mechanisms driving assembly of neural circuits, which may inform interventions to promote neuronal repair.

811C Developmental axon guidance cues are critical for adult neuronal survival and function *Aarya Vaikakkara Chithran*, Douglas Allan, Timothy O'Connor Department of Cellular and Physiological Sciences, University of British Columbia, Vancouver, BC, Canada.

One of the most extraordinary characteristics of the nervous system is the complexity of its neural circuits. Although there are many mechanisms involved in shaping the pattern of these connections, one of the most important events is the guidance of axons to their specific targets. Model organism research in the past several decades has advanced our understanding of the function of numerous guidance cues, their receptors and their underlying signaling cascades. More recently it has become clear that after functional circuits have been established, many neurons continue to express developmental guidance cues. The expression of these genes in the adult indicates that there are likely additional roles for them beyond the initial phase of neuronal process outgrowth, growth cone navigation and target innervation.

To test this hypothesis, we performed an RNAi screen against axon guidance genes expressed in the adult nervous system. Using available databases, 151 axon guidance genes were identified that are expressed in the adult *Drosophila* nervous system. 44 genes were prioritized based on their higher expression profiles and previously known roles in neuronal pathfinding. We knocked down the expression of each of these genes using spatial and temporal control of the GAL4-UAS system and identified 15 genes that are required for adult survival. Knockdown of these 15 guidance cues also caused motility defects.

To understand the impact of axon guidance gene knockdown at a cellular level, we examined well-established circuits such as the olfactory system and motoneuron circuits in adult *Drosophila*. Knocking down Fasciclin-3, an Ig containing homophilic cell adhesion molecule, led to neuronal death in the adult olfactory system. This phenotype was rescued

by overexpressing p35, an anti-apoptotic protein. Knocking down Semaphorins and Plexins, members of the canonical repulsive guidance signalling pathway, resulted in loss of motoneurons. Currently, we are examining the impact of Semaphorin/ Plexin double knockdowns to understand the cellular and molecular mechanism responsible for these phenotypes. We are also examining the impact of Semaphorin/ Plexin knockdown on the olfactory circuit and neuromuscular junctions.

Taken together, we believe that the continued expression of axon guidance genes in the adult is critical for the maintenance of neural circuits in the mature nervous system.

812A Target-independent visual map formation *Egemen Agi*[1], Charlotte B. Wit[1], Eric Reifenstein[2,3], Max von Kleist[2,3], P. Robin Hiesinger[1] 1) Division of Neurobiology, Institute for Biology, Freie Universitaet Berlin, Germany; 2) Systems Medicine of Infectious Disease (P5), Robert Koch Institute, Berlin, Germany; 3) Bioinformatics (MF1), Robert Koch Institute, Berlin, Germany

The fly visual map represents ~800 neighboring points in space as synaptic ensembles (cartridges) in the lamina of the fly brain. These 800 visual axes are represented by six photoreceptor axons (R1-R6) in each cartridge, where R1-R6 provide pooled presynaptic input onto their main postsynaptic partner lamina cells L1-L3. Due to the arrangement of separate light-sensing elements that receive input from different visual axes in each single ommatidium of the fly eye, R1-R6 axons from six different ommatidia have to grow towards a shared cartridge in the lamina, a principle called 'neural superposition'. In effect, six times 800 R1-R6 axons synchronously grow away from their original arrival points in the lamina by elongating their growth cones orthogonally to their axons in a sorting plane. At the time of growth cone sorting, the postsynaptic L cells are present in the lamina in an apparent target grid. Surprisingly, genetic ablation of L cells does not affect early pattern formation, growth cone elongation nor the initial formation of the neural superposition pattern. Only after superposition patterning through R1-6 growth cones alone, the fronts of all elongated growth cones are stabilized by the 'target grid' in an N-Cadherin-dependent manner. To understand how initial neural superposition pattern formation is established by six times 800 growth cones in the absence of any other cell type, we performed non-invasive two-color two-photon live imaging and developed a computational model of the process based on photoreceptor filopodial dynamics. Both data and model reveal a dynamic pattern formed by the 'heels' (back ends) of the elongating growth cones with shifting membrane densities throughout the sorting process. Front filopodia predominantly sample the space between the highest heel densities, i.e. the valleys of the heel density landscape. In turn, the weighted directions and lifetimes of front filopodia predict the subsequent growth cone elongation vectors. We conclude that six times 800 photoreceptor growth cones self-organize into the neural superposition pattern by separating growth cone front and heel patterns, with the former appearing to chase the latter. This self-organization process can be explained and modeled based on a minimal number of guidance cues by largely relying on intrinsic and dynamic cell and tissue properties.

813B Temporal regulation of nicotinic acetylcholine receptor subunits supports central cholinergic synapse development in *Drosophila* *Justin Rosenthal*[1,2], Jun Yin[1], Jimmy Lei[1], Anupama Sathyamurthy[1], Jacob Short[1], Caixia Long[1], Emma Spillman[3], Chengyu Sheng[1], Quan Yuan[1] 1) National Institutes of Health, Bethesda, MD; 2) University of Maryland-College Park, College Park, MD; 3) University of California-San Diego, San Diego, CA

The construction and maturation of the postsynaptic apparatus are crucial for synapse and dendrite development. The fundamental mechanisms underlying these processes are most often studied in glutamatergic central synapses in vertebrates. Whether the same principles apply to excitatory cholinergic synapses, such as those found in the insect central nervous system, is not known. To address this question, we investigated a group of projection neurons in the *Drosophila* larval visual system, the ventral lateral neurons (LNvs), and identified *Dα1* and *Dα6* as the main functional nicotinic acetylcholine receptor (nAchR) subunits in the larval LNvs. Using morphological analyses and calcium imaging studies, we demonstrated critical roles of these two subunits in supporting dendrite morphogenesis and synaptic transmission. Furthermore, our developmental expression profiling and endogenous tagging approaches identified distinct transcriptional controls over the two subunits resulting in the up-regulation of *Dα1,* which is suppressed by elevated presynaptic activity, and down-regulation of *Dα6* during larval development. Additional functional analyses of synapse formation and dendrite dynamics further revealed a close association between the temporal regulation of these two nAchR subunits and their sequential requirements during cholinergic synapse maturation. Finally, preliminary screens have identified candidate regulatory factors coordinating nAchR expression and postsynaptic activity, including the transcription factor and activity-regulated gene Hr38 as well as the nAchR-specific molecular chaperone NACHO. Together, our findings highlight how transcriptional control of nAchR subunits is a core element of developmental and activity-dependent regulation of central cholinergic synapses, which is likely dependent on both transcriptional and post-translational processes.

814C Differential expression of the roundabout 3 (Robo3) guidance receptor regulates interneuron dendrite morphogenesis in *Drosophila melanogaster* somatosensory circuit development *Jake Henderson*, Ellie Heckscher University of Chicago

For any animal to sense and interact with their environment, it is critical their neural circuits develop properly. The development of functioning neural circuits requires each neuron to correctly position its axonal and dendritic arbors and establish synaptic connections with their correct partners. Within neural circuits, interneurons are the most numerous cell type and are required for complex processing of sensory stimuli. Interneurons are an under investigated cell type due to the lack of tools and knowledge regarding neural circuit architecture within the CNS. In *Drosophila*, recent studies have clarified unique synaptic connections for many circuits, however how these connections are determined for interneurons is unclear. Temporal transcription factors and guidance cues are known to regulate neuronal development, however the role of these molecular factors has not been deeply investigated in interneuron dendrite development, synaptic partner selection, and circuit formation. We hypothesize that differential guidance receptor expression establishes specific dendritic morphologies and synaptic connections within interneurons born from the same stem cell. We investigated this hypothesis using the *Drosophila melanogaster* Neuroblast 3-3 stem cell lineage as a model. The NB3-3 lineage produces two cohorts of interneurons that create synaptic connections with two different sensory circuits: vibrational and proprioceptive. During development, the sensory neurons position their axons first at different locations within the ventral nerve cord. The two cohorts of even-skipped(+) lateral (EL) interneurons are then required to position their dendrites appropriately to receive the sensory input. We investigated the role of guidance receptors in interneuron dendrite morphogenesis by examining the expression pattern of Roundabout receptors within EL interneurons. We also examined the necessity and sufficiency of the Roundabout 3 (Robo3) receptor by manipulating its expression. These experiments revealed that Robo3 is involved in regulating EL interneuron dendrite position and therefore is a potential important factor in determining circuit wiring. This work will also enable future research to investigate the broad conservation of neuronal circuit specification and development of therapies for neurodevelopmental pathologies in which circuit wiring is disrupted.

815A Promiscuous wiring via variable spatial sampling of an orderly array *Emma Thornton-Kolbe*, Maria Ahmed, E. Josie Clowney University of Michigan, Ann Arbor MI

The patterns of neuronal connectivity determined by development generate much of the computational power of the central nervous system. In brain regions devoted to sensory processing, sparse, combinatorial connectivity expands perceptual capacity. In the *Drosophila* mushroom body, sparse combinatorial connectivity between projection neurons carrying olfactory information from the periphery and Kenyon cells results in separable coding of many diverse scents and thus underlies successful associative learning. Each Kenyon cell (KC) receives input from a handful of projection neuron (PN) types through claw- like dendrites which each enwrap a single projection neuron bouton. Our lab has demonstrated that Kenyon cells determine connectivity density through the number of claws they produce, which are invariant when KC population is increased or reduced. Rather, PN bouton number changes to accommodate the changes in postsynaptic KCs. Here, we ask how the combinations of PN inputs to KCs are determined. Two possible sources of variation in projection neurons connections to Kenyon cells are molecular and spatial variation. By describing levels of transcriptional variation among projection neurons and Kenyon cells and the spatial relationships among these cells in connectomic data, we are testing the relative importance of these factors. Insights into the development of the mushroom body calyx will add to the existing knowledge of the diverse ways in which neuronal connectivity is controlled during development to produce functional circuits.

816B Codes of cell surface proteins coordinate stochastic and deterministic cell fates during *Drosophila* color vision circuit assembly *Yu-Chieh David Chen*, Claude Desplan New York University, Department of Biology, New York, NY

How vast numbers of neurons are specified into correct cell fates and connected with their proper targets during development represents a fascinating area of developmental neuroscience. Mechanisms of stochastic and deterministic cell specification programs to achieve neuronal diversity have been extensively studied. A number of axon guidance molecules have also been identified. However, little is known about the coordination between neuronal specification and specific connectivity patterns, especially when two synaptic partners undergo two different modes of cell specification (stochastic vs. deterministic). In the fly retina, pale (**p**) and yellow (**y**) subtypes of color photoreceptors (R7 and R8) are stochastically specified, whereas their synaptic partners in the optic lobe are produced through highly deterministic programs. How do stochastically determined **p** vs. **y** R7 and R8 find their respective targets that are deterministically specified in the optic lobes?

By focusing on **p**/**y** R7 and their main downstream target **p**/**y** Dm8 neurons, we and others have recently identified a pair of DIPs and Dprs, members of an interacting network of immunoglobulin superfamily proteins, as critical regulators of the synaptic connection between **y**R7 that expresses Dpr11 and **y**Dm8 that expresses DIPγ. We thus hypothesize that different pairs of cell adhesion molecules can mediate the matching of other synaptic partners. We aimed to uncover such molecular code for the pairing of **p**R7 and **p**Dm8. It is unlikely to be achieved via another DIP-Dpr pair since our recent single-cell RNA sequencing (scRNAseq) data showed no other DIPs expressed in Dm8s. To identify candidate molecule(s) that mediate **p**R7-**p**Dm8 connectivity, we bioinformatically split our recent scRNAseq transcriptomic profile of the Dm8 cluster into DIPγ^{+} (**y**Dm8s) and DIPγ^{-} (**p**Dm8s) neurons during development. We found that Beat-IIIc, a

member of Beat family proteins, is specifically expressed in **p**Dm8s cells during the developmental stage of synaptic partner pairing. Members of the Beat family and Side proteins form a receptor-ligand system to control axonal targeting. Since there are 14 Beat and 8 Side members, we aim to survey the expression pattern of all the Sides and look for their expression in **p**R7s. Preliminary experiments suggest Side-V is a promising candidate as it is only expressed in a subset (presumably **p**) of R7 terminals. We have generated CRISPR mutant alleles of these candidates and will be presenting our functional analyses of these candidate molecules in regulating synaptic partner matching. Overall, our work has uncovered novel molecular mechanisms regulating synaptic pairing and probes the fundamental principles underlying the propagation of stochastic cell fate choices during circuit assembly.

817C Investigation of the tRNA modifying enzyme, TRMT1, in neurodevelopment *Sara Ríos Méndez*[1], Kimberly Rose Madhwani[2], Ámbar Delgado[3], Jennifer Dumouchel[4], Kate O'Connor-Giles[3,5] 1) NIH Post-Baccalaureate Research Education Program (PREP), Brown University, Providence RI; 2) Neuroscience Graduate Training Program, Brown University, Providence, RI; 3) Department of Neuroscience, Brown University, Providence, RI; 4) Molecular Pharmacology & Physiology Graduate Training Program, Brown University, Providence, RI; 5) Carney Institute for Brain Science, Brown University, Providence, RI

The dynamic regulation of global gene expression is required for proper development and function of the nervous system. Emerging work reveals that dysregulation of global gene expression can result in a myriad of neurodevelopmental and degenerative disorders. Specifically, transfer RNAs (tRNAs), which act as adaptors to decode messenger RNA and deliver the correct amino acid to growing polypeptides, undergo a variety of post-transcriptional modifications that are crucial for tRNA folding and stability, thereby ensuring accurate protein synthesis. tRNA methyltransferase 1 (TRMT1) is one of two mammalian orthologs of yeast tRNA methyltransferase, Trm1. TRMT1 dimethylates the guanosine nucleotide at position 26 of most tRNAs to yield the N2,N2-dimethylguanosine (m2,2G) modification. Frameshift mutations in human TRMT1 underlie autosomal recessive intellectual disability in multiple families, suggesting that guanosine-26 demethylation of tRNAs plays an important role in proper neurodevelopment and function. Potentially overlapping roles for TRMT1 and TRMT1-L complicate mammalian studies so we generated a model of TRMT1 in Drosophila, which encodes a single Trm1 homolog. Our preliminary studies suggest a role for TRMT1 in promoting synapse formation. We will report our latest findings on TRMT1's *in vivo* role in nervous system development and function. Further study into TRMT1 will provide insight into the important role of tRNA modification in regulating gene expression in the nervous system.

818A Neurodevelopmental role of a tRNA methyltransferase implicated in intellectual disability *Kimberly Rose Madhwani*[1], Sara Ríos Méndez[2], Caley Hogan[3], Jennifer L. Dumouchel[4], Jenna Lentini[5], Dragony Fu[5], Kate M. O'Connor-Giles[6,7] 1) Neuroscience Graduate Training Program, Brown University, Providence, RI; 2) NIH Post-Baccalaureate Research Education Program (PREP), Brown University, Providence RI; 3) Laboratory of Genetics, University of Wisconsin-Madison, Madison, WI; 4) Molecular Pharmacology & Physiology Graduate Training Program, Brown University, Providence, RI; 5) Department of Biology, University of Rochester, Rochester, NY; 6) Department of Neuroscience, Brown University, Providence, RI; 7) Carney Institute for Brain Science, Providence, RI

Gene regulation at multiple levels is critical for nervous system development and function. A number of mutations leading to global misregulation of gene expression disproportionately affect the nervous system, resulting in both neurodevelopmental and degenerative disorders. Emerging work demonstrates that post-transcriptional modification of transfer RNAs (tRNAs) regulates tRNA stability and codon-anticodon pairing and, thus, translation rate and fidelity. ALKBH8 is one of two metazoan homologs of the yeast tRNA methyltransferase TRM9. Mutations in the highly conserved human ALKBH8 were recently found to cause intellectual disability in three families. However, ALKBH8's role in the nervous system is unknown. In yeast, TRM9 methylates uridines in the wobble position of the anticodon loop to reinforce cognate codon-anticodon pairings, resulting in increased translation of mRNAs enriched for cognate codons. We generated *ALKBH8* null mutants and analyzed tRNA post-transcriptional modifications in the brain by mass spectrometry. We observed a complete loss of wobble uridine methylation in *Drosophila ALKBH8* null mutants, consistent with prior findings in mammals. We next investigated a role for ALKBH8 in nervous system development and found that ALKBH8 attenuates synaptic growth. In addition, we found that *ALKBH8* mutants exhibit increased levels of and sensitivity to reactive oxygen species (ROS). ALKBH8 is required to methylate tRNA-selenocysteine, which enables the incorporation of selenocysteine into selenoproteins. Selenoproteins are potent regulators of oxidative stress, and we have found that blocking selenoprotein synthesis by other means also results in synaptic overgrowth. Interestingly, oxidative stress has been shown to manifest in synaptic overgrowth. To determine if increased ROS and synaptic overgrowth in *ALKBH8* mutants are causally linked, we treated *ALKBH8* null animals with the antioxidant N-acetylcysteine amide and found that synaptic growth is partially rescued. These findings support a model in which ALKBH8 regulates synaptic growth through its role in regulating ROS via methylation of tRNA-selenocysteine, and reveals antioxidants as a potential therapeutic treatment for individuals with ALKBH8-linked intellectual disability.

819B Long-range temporal patterning of neuroblasts in the developing *Drosophila* medulla couples neurogenesis to circuit assembly *Teddy Erclik*, Urfa Arain, Maliha Islam, Priscilla Valentino University of Toronto - Mississauga

The *Drosophila* medulla, which is the largest neuropil in the optic lobe, is as an excellent model system in which to study the mechanisms that regulate neurogenesis. Its 40,000 neurons, which comprise over 90 cell types, are generated from a neuroepithelial crescent termed the outer proliferation center (OPC). Beginning at the onset of the third larval instar, and continuing for two days, a proneural wave converts neuroepithelial cells into neuroblasts (NBs), which subsequently divide asymmetrically to generate the neurons and glia of the medulla cortex. It has previously been shown that two axes of positional information act on OPC NBs to generate neural diversity. In the temporal axis, a cascade of five genes--Hth, Ey, Slp1, D and Tll--are sequentially expressed in each of the medulla NBs as they age. In the spatial axis, the OPC crescent from which the NBs are generated is sub-divided into eight compartments (patterned by four genes: Vsx1, Optix, Rx, and Hh). Distinct neuronal types are generated by NBs based on their spatio-temporal address. Here, we describe a third patterning axis that further diversifies neuronal fates in the medulla. We show that the neuroepithelium from which the NBs are generated is itself temporally patterned by the expression of five genes--Imp, Syp, Chinmo, Mamo and E93--over the two-day period of neurogenesis. This long-range temporal patterning of the neuroepithelium confers NBs from the same spatio-temporal address with unique identities based on the developmental time that they are generated. Using clonal- and EdU-birthdating analyses, we show that NBs in the Vsx1-Hth spatio-temporal window surprisingly generate not one, but four distinct neuronal types over the course of neurogenesis. These neurons (Tm23, TmY15, Pm3 and TmY12) are generated in distinct developmental windows and their specification is dependent on the activity of the long-range temporal factors; in *syp* mutants, early-born TmY15 neurons are expanded in number, whereas late-born Pm3 neurons are lost. We further show that long-range temporal patterning extends beyond the Hth temporal NB window to concurrently pattern NBs in the Ey, Slp1 and D temporal windows as well. Finally, we demonstrate that the birthdate of medulla neurons correlates with their final anterio-posterior position in the adult cortex and propose that long-range temporal patterning functions as a mechanism to couple neurogenesis with circuit assembly.

820C Coordinated control of neuronal differentiation and wiring specificity by a sustained code of transcription factors *Mehmet Neset Ozel*[1], Claudia Skok Gibbs[1,2], Isabel Holguera[1], Richard Bonneau[1,2], Claude Desplan[1] 1) Department of Biology, New York University, New York, NY; 2) Flatiron Institute, New York, NY

Cascades of transcription factors (TF) that are transiently expressed in neural stem cells are responsible for generating the enormous diversity of cell types in nervous systems. However, the gene-regulatory mechanisms that establish and maintain these cell fates in postmitotic neurons and instruct their specific morphology, connectivity and physiology remain largely unclear. Using a large scRNA-seq atlas of the *Drosophila* optic lobes (Özel et al. 2020, *Nature*), we tracked ~200 neuronal types across 6 stages and found that each one stably expresses a unique combination of 95 TFs (~10 per cell type) that are maintained throughout development to adulthood. We hypothesized that these function as “selector” TFs that are activated in each neuron immediately after their birth and function as top-level regulators of all type-specific gene expression thereafter. Through genetic gain- and loss-of-function experiments, we show that modification of these selector TF codes is sufficient to induce predictable switches of identity between various optic lobe neurons. For instance, *pdm3* is necessary and sufficient to determine the fate choice between Tm2 and Tm4 neurons. In addition to their morphological identity, scRNA-seq of perturbed neurons revealed that such conversions are also transcriptomically complete. Similarly, ectopic expression of Vsx genes in Mi15 neurons not only leads their complete morphological conversion to Dm2, but also a loss of their aminergic identity. Thus, continuously maintained TF codes instruct both the adult terminal features and the type-specific development of optic lobe neurons.
To understand the mechanisms that control brain wiring downstream of selector TFs, we combined scRNA and scATAC-seq (chromatin accessibility) datasets to build computational models of gene regulatory networks using Inferelator 3.0 (Gibbs et al. 2021, *BioRxiv*). Networks inferred at mid-pupal stages suggest that selector TFs interact with hormone-responsive TFs to activate a large repertoire of cell-surface proteins in each neuron prior to synapse formation. For instance, we show that Netrin receptor Frazzled, downstream of *pdm3*, specifically mediates the unique ‘forking’ of Tm2 dendrites: overexpression of *fra* in Tm4 neurons is sufficient to produce this feature.
Overall, our results provide a unified framework of how specific fates are maintained in postmitotic neurons, and open up new avenues to understand synaptic specificity through gene regulatory networks.

821A Persistence of courtship behavior neurons from larval to adult life in *Drosophila* *Sofia Leone*, Julia Duckhorn, Sofia Altamirano, Troy Shirangi Villanova University, Villanova, PA

The *dissatisfaction* gene (*dsf*) in *Drosophila* is required for development of discrete 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. The developmental origin of the ddag neurons is currently unclear. Here, we used the Flp-Switch system to conditionally immortalize gfp expression in *dsf*-expressing neurons of the larva and found that a subset of these neurons become the ddag neurons of the adult. During pupal life, a subset of *dsf*-expressing abdominal interneurons present in the larval central nervous system of both sexes gain expression of the sex determination gene, *doublesex*. We hypothesize that these larval neurons sexually differentiate during pupal development to contribute to abdominal courtship behaviors in the adult. Our results suggest that the neural circuits for

courtship behaviors in the adult arise in part from larval interneurons that are repurposed during pupal life.

822B Differentiation signals from glia are fine-tuned to set neuronal numbers during development *Anadika Prasad*, Matthew Bostock, Ines Lago-Baldaia, Zaynab Housseini, Vilaiwan Fernandes University College London

The number of neurons formed during development need to be tightly regulated to form functional neural circuits. Known strategies include regulating the number of neurons that are formed or that survive during development. Here, we study how neuronal numbers are regulated in the lamina neuropil of the visual system. The lamina is comprised of ~800 units or columns. Each column consists of six precursors, of which only five differentiate into the five lamina neuron types (L1-L5). The extra precursor is eliminated by apoptosis. This process is highly stereotyped with neuronal differentiation and apoptosis occurring invariantly in the same cell positions in each column. We asked how exactly five lamina neurons (and neuron types) differentiate from a pool of six precursors. Previously we showed that L1-L4 neurons are induced to differentiate by wrapping glia. Therefore, we focused on the extra precursor and the differentiating L5s. We uncovered that differential Hedgehog signaling levels along the length of columns pattern lamina precursors, such that the 2 precursors experiencing the lowest levels of signaling are specified as L5s. Thus, twice as many precursors are specified as L5s than undergo differentiation normally. We showed that in response to photoreceptor-derived Spitz, a glial population, the outer chiasm giant glia (xg^{O}), secrete multiple ligands (Spitz and Collagen IV alpha 1) to induce L5 differentiation. Moreover, we found that the newly-induced L5 neurons antagonize differentiation signals from reaching the extra precursors, which as a result undergo apoptosis. Therefore, our results indicate that newly induced neurons limit the availability of glial differentiation signals which sets the number of L5 neurons in each lamina column. Furthermore, our work highlights how stereotyped patterns of programmed cell death in the lamina arise from extrinsic signals which reliably pattern the development of the nervous system.

823C Dorsal-Ventral Patterning of the Developing *Drosophila* Medulla *Priscilla Valentino*[1,2], Ted Erclik[1,2] 1) University of Toronto, Toronto, Ontario, Canada; 2) University of Toronto: Mississauga, Mississauga, Ontario, Canada

In the *Drosophila* visual system, photoreceptors in the compound eye project visual information to neurons in the underlying optic lobe for processing. The largest neuropil of the optic lobe is the medulla, which mediates motion and colour processing, and is comprised of over 90 cell types. The medulla develops from a larval crescent of neuroepithelial (NE) cells termed the outer proliferation centre (OPC). In the 3rd instar larva, the NE cells of the OPC are converted into neuroblasts, which then asymmetrically divide to generate the 90 types of medulla neurons. Previous studies have shown that two axes of positional information are required for neuronal specification. In the temporal axis, 5 transcription factors are sequentially expressed in the medulla neuroblasts as they age. In the spatial axis, the OPC is patterned into 5 spatial compartments by the non-overlapping expression of 3 homeobox transcription factors; Vsx1 in the center of the crescent, Optix in the adjacent arms and Rx in the tips. Thus, distinct neuronal types can be assigned unique spatio-temporal birth addresses based on the spatial compartment and temporal window from which they were born. Surprisingly, it has also been observed that neurons with identical spatio-temporal birth addresses can assume different fates based on whether they are born in the dorsal or ventral half of the OPC, suggesting that the dorsal and ventral halves of the OPC crescent are also spatially patterned. Here, using a technique involving micropipette-based cell isolation and RNA-seq, we identify 4 transcription factors that are differentially expressed in the dorsal and ventral halves of the OPC NE; *salm* and *salr* are expressed in all dorsal OPC cells, whereas *disco* and *disco-r* are expressed in all ventral cells. We show that the expression patterns of these genes meet to form a sharp dorsal-ventral boundary in the Vsx1 region of the OPC. We further demonstrate that *salm*/*salr* and *disco*/*disco-r* negatively cross-regulate each other in the OPC NE to maintain this dorsal-ventral compartment boundary. Loss- and gain-of-function experiments are currently underway to determine whether these genes contribute to medulla neuronal specification. It is anticipated that investigating the neurogenic role of these genes in the medulla will further our understanding of how neural diversity is generated at the intersection of temporal and spatial inputs.

824A Developmental patterns of the *Drosophila* visual projection neurons *Rana Eldanaf*[1], Claude Desplan [1, 2] 1) Center for Genomics and Systems Biology, New York University Abu Dhabi, United Arab Emirates; 2) Department of Biology, New York University, New York, NY 10003, USA.

The *Drosophila* visual system is a powerful model for studying diverse aspects of neural circuit development. It is comprised of four neuropils, each essential for processing various visual stimuli. Projection neurons in the lobula (the lobula columnar neurons-LCNs), are thought to integrate different visual cues, send that information to the central brain leading to specific behavioral outputs in response to these stimuli. LCNs comprise ~20 morphological and functional subtypes. Interestingly, each LCN subtype forms a highly specific, neighboring yet non-overlapping synaptic domains in the brain called optic glomeruli. While there have been functional investigations of several LCN circuits, we know very little about how these are established. We screened Janelia Gal4, split-Gal4 and MiMIC lines to identity driver lines that are expressed in specific LCNs throughout development. We visualized axonal and dendritic growth using ;10xUASmyr::GFP; reporter line. To address wiring specificity, we analyzed transcriptomic data using scRNA-Seq to identify LCN specific markers. We identified a population of LCNs at larval stages of the optic lobe, which express the

transcription factors toy and acj6. Our results indicate that they belong to three LCN subtypes previously identified to encode looming and the onset of edge. The high specificity of expression in this line provides an ideal tool for further developmental characterization of these neurons, as well as to understand how their highly precise patterns of axon-target matching occurs during development.

825B Loss of the GARP but not EARP complex drives Golgi sterol overload during dendrite remodeling *Caitlin O'Brien*[1,2], Susan Younger[1,2], Lily Jan[1,2], Yuh Nung Jan[1,2] 1) Howard Hughes Medical Institute; 2) University of California San Francisco

Membrane trafficking pathways are essential to sculpting neuronal morphology. The closely related GARP (*G*olgi-*A*ssociated *R*etrograde *P*rotein) and EARP (*E*ndosome-*A*ssociated *R*ecycling *P*rotein) complexes are conserved membrane tethers that function in the secretory and endolysosomal pathways. These complexes share the proteins Vps51, Vps52, and Vps53, and each contain a complex-specific protein: Vps54 in GARP and Vps50 in EARP. Several mutations in the core components (*Vps51* and *Vps53*) are associated with neurodevelopmental disorders, but a precise role for these complexes in neurodevelopment is still unclear. We generated CRISPR knockout *Drosophila* of the shared component *Vps53*, and the complex-specific components *Vps50* and *Vps54* (also known as *scattered*). We find that both complexes are required for dendrite morphogenesis at a specific time during development. Larval neurons are largely spared, but alterations in endolysosomes and dendrite morphology emerge during remodeling of peripheral sensory neurons during pupation. We also observe accumulation of sterol specifically in GARP-, but not EARP-deficient neurons. Previous studies of GARP-deficient cells found that sterol accumulates in lysosomes. To our surprise, we find that sterols accumulate at the trans-Golgi network (TGN), not in lysosomes, in *Vps54*$^{KO/KO}$ *Drosophila* neurons. As sterol auxotrophs, *Drosophila* obtain sterol from dietary sources only and therefore may utilize additional mechanisms to transfer sterol from the endolysosomal to the secretory pathway after uptake. Targeting genes that regulate sterols and related lipids at the TGN can modulate the *Vps54*$^{KO/KO}$ phenotype. Specifically, overexpressing the oxysterol binding protein (Osbp), which transfers sterol between the endolysosomal and secretory pathways at multiple membrane contact sites, exacerbates the dendrite regrowth defect of *Vps54*$^{KO/KO}$ neurons, while a null allele of Osbp partially rescues it. This suggests that the activity of Osbp may be upregulated in *Vps54*$^{KO/KO}$ neurons and that the accumulation of excess sterol at the TGN is, at least in part, responsible for inhibiting dendrite regrowth. These studies begin to functionally distinguish the GARP and EARP complexes in neurodevelopment and indicate that both membrane trafficking and lipid transfer pathways are essential for proper dendrite growth.

826C Genetic mechanisms underlying the development and distribution of Dm4 neurons in the *Drosophila* medulla *Urfa Arain*[1], Ted Erclik[1, 2] 1) Cell & Systems Biology, University of Toronto, Toronto, Ontario, Canada ; 2) Biology, University of Toronto, Mississauga, Ontario, Canada

The *Drosophila* compound eye is comprised of an array of photoreceptor units that send visual stimuli to the processing centers of the brain. Colour and motion processing occurs in the medulla, which is made up of repeating columnar units that are innervated by over 90 neuronal cell-types. During brain development, a subset of medulla neurons migrate from their spatially restricted birth locations to reach their final positions in the adult retinotopic circuit. To date, little is known about the mechanisms underlying this movement of medulla neurons. To elucidate the mechanisms that regulate this process, we have focused on the migration of a single neuronal cell-type, Dm4. There are approximately 40 Dm4 neurons per medulla, each of which cover ~20 columns across the antero-posterior and dorso-ventral axes of the adult retinotopic field. A single Dm4 neuron sends its axon into the M6 layer of the medulla neuropil, and forms dendritic arbors in the superficial M3 layer to make synaptic contacts with lamina monopolar cells (L3). To understand how these circuits are established, we generated a Dm4-specific split-Gal4 driver that allows us to track the relationship of Dm4 movement with morphological changes during development. We find that Dm4 neurons are born in the mid-third-instar larval brain as a cluster of cells in the medial and ventral region of the developing medulla cortex. Subsequently, during pupal development, Dm4 neurons move across the antero-posterior and dorso-ventral axes. We find that Dm4 neurons initiate their movement across the antero-posterior axis at ~25h APF, followed by their dorso-ventral migration 5 hours later at ~30h APF. We show that Dm4 axons project to their target regions in the medulla neuropil well before migration is initiated, whereas dendritogenesis occurs during migration and is completed by 48h APF. An RNAi-based screen for genes required for Dm4 migration identified the transcription factor Traffic Jam (Tj) as a critical regulator of the process. Loss of *tj* in Dm4 neurons results in a ventromedial cluster of cells in the adult medulla that failed to migrate across both the antero-posterior and dorso-ventral axes. *tj*-mutant Dm4 neurons project to the correct target layers in the medulla, but exhibit disorganized dendritic projections in the M3 layer. Strikingly, single-cell clones of *tj*-mutant Dm4s reveal regional differences in the severity of these defects; cells fated to move a larger distance from their birth location exhibit greater dendritic defects compared to those with shorter migration trajectories. Taken together, our data support a model in which neuronal migration is required for neurons to form proper dendritic connections within their target columns.

827A Investigating the role of VAPB in axonal ER and motorneuron development and degeneration Elizabeth Anderson Case Western Reserve University

Smooth ER (endoplasmic reticulum) forms a continuous network inside of each motorneuron, dominating the intracellular landscape and extending through each axon from root to tip. Smooth ER acts as a source and sink of Ca^{+2} for organelles and tethers itself to other organelles, all lending to the hypothesis that it could be dynamically communicating and trafficking intracellular information, like a "neuron within a neuron". Recent findings suggest that VAPB (vesicle-associated membrane protein [VAMP]-associated protein B) regulates smooth ER morphology and function in cultured cells. A highly conserved portion of VAPB can also be cleaved to act as an extracellular ligand. Intriguingly, this domain can harbor a point mutation (P58S) causative of amyotrophic lateral sclerosis (ALS). The complete molecular mechanisms linking VAPB to ER and motorneuron development and degeneration remain elusive. We will investigate these interactions using a simple *Drosophila* Crispr-mediated knockout model that allows us to knock back in both GFP-tagged rescue and P58S VAPB constructs. Excitingly, the VAPB knockout I engineered seems viable. In conjunction with new adult neuromuscular junction (NMJ) dissection techniques, this uniquely poises us to track synaptic development and VAPB localization at the NMJ *in vivo* from larvae to adult. We will observe gross ER morphology and function by crossing these mutants with existing lines that mark ER, calcium signaling, and autophagosomal trafficking. Flight behavior assays will measure motorneuron activity. We hypothesize, due to prior *in vitro* studies, that VAPB knockout and VAPB P58S mutant *Drosophila* will exhibit deficiencies in VAPB localization and ER morphology and function at the NMJ. We further hypothesize that this will correlate with deficits in motorneuron development, and ultimately degeneration and altered flight behaviors.

828B The Role of Thrombospondin in Neuromuscular Junction Development and Function *Grace Woods*[1], Karli Corey[1], Sonya Lee[1], Eve Lowenstein[2], Isa Maxwell[1], Asia Wooten[3], Allie Osgood[4], Luke Rotello[5], Daniela Mendoza Ortiz[6], Norma Velazquez Ulloa[1] 1) Department of Biology, Lewis & Clark College, Portland, OR; 2) PMCB Graduate Program, OHSU, Portland, OR; 3) Medical School Program, OHSU, Portland, OR; 4) National Human Genome Research Institute, NIH, Bethesda, MD; 5) Biochemistry and Molecular Biology Program, Lewis & Clark College, Portland, OR; 6) University of Turku, Turku, Finland

Thrombospondin (TSP) is an extracellular matrix glycoprotein that plays a role in synaptogenesis at glutamatergic synapses in the mammalian brain. While there are 5 TSP genes in humans, there is a single homologous gene in *Drosophila melanogaster* (D-TSP) and there is conservation in the protein domains involved in TSP's function in synaptogenesis. It has not been investigated if D-TSP plays a role in synaptogenesis in *D. melanogaster*. Here we determined if D-TSP modulates synaptogenesis and locomotor behavior in the *D. melanogaster* third instar larval NMJ. We hypothesized that D-TSP would be necessary for normal NMJ formation and locomotor behavior. We used the GAL4-UAS system to knock down D-TSP in neurons with two different RNAi lines and quantified features of the NMJ structure and locomotor behavior in larvae with normal or decreased D-TSP expression. Both RNAi experiments showed no significant survival differences between the parental lines and the cross. For the anatomical analysis, we took images of NMJs from muscle 4 of segments A3-4 in female third instar larvae from both RNAi crosses. We manually counted the number of boutons, number of branches, number of branch points, and number of islands of each NMJ, and generated a complexity index comprising the number of branches, number of branch points, and number of islands. Our preliminary results from experiments with one of the RNAi lines suggest an increase in NMJ complexity when D-TSP is knocked down in neurons. We also calculated the polygon area for each NMJ, and found no differences in the D-TSP knockdowns. We are currently normalizing our morphological data by muscle area, increasing the sample size of NMJs for the first RNAi line, and completing our anatomical analysis of the second RNAi line. To investigate locomotor behavior, we took videos of male and female larvae moving in a gridded arena, and analyzed the larval trajectories for distance, velocity, curvature, and zones (a measure of how far larvae move from their point of origin). We found that TSP knockdown larvae from the second RNAi line exhibited a significantly higher distance travelled compared to control larvae. We are in the process of validating the knockdowns of D-TSP by RT-qPCR. In the future, we will analyze additional behaviors, such as head-turning and body contractions.

829C Investigating roles of conserved domains in the calcium channel subunit $\alpha_2\delta$-3 during synapse development *Marina Bostelman*, Heather Broihier Case Western Reserve University, Cleveland, OH

Synapses are highly specialized for neurotransmitter signaling, yet activity-dependent growth factor release also plays critical roles at synapses. While efficient neurotransmitter signaling relies on precise apposition of release sites and neurotransmitter receptors, molecular mechanisms enabling high-fidelity growth factor signaling within the synaptic micro- environment remain obscure. At the *Drosophila* neuromuscular junction (NMJ), the highly evolutionarily conserved auxiliary calcium channel subunit $\alpha_2\delta$-3 promotes an activity-dependent, autocrine Bone Morphogenic Protein (BMP) signal by functioning as an extracellular scaffold, thereby regulating synapse density, structure, and function. Furthermore, $\alpha_2\delta$-3's promotion of BMP signaling is independent of its canonical role in the localization of the pore-forming α_1 calcium channel subunit, Cacophony. How these functions are separated, and what roles several evolutionarily conserved protein domains within $\alpha_2\delta$-3's structure play in each of these functions, is currently unknown. This question is being addressed by utilizing the CRISPR-Cas9 gene editing system to generate *Drosophila* lines containing deletions or modifications of highly conserved key motifs within $\alpha_2\delta$-3. Assessing NMJ morphology and function in these

mutants at embryonic and larval stages will provide insight into the role of these motifs in synapse formation throughout early *Drosophila* development.

830A Inhibitors of BMP signaling during synapse development in *Drosophila melanogaster* Kendall Cook[1], Cameron Rodriguez[1], Joseph Peters[1], Polina Yagusevich[1], Emily Januck[1], Kaitlin Tortorete[1], Amin Ghabrial[2], *Pam Vanderzalm*[1] 1) John Carroll University, Cleveland, OH; 2) Columbia University, New York City, NY

The canonical Bone Morphogenic Protein (BMP) pathway helps coordinate the growth and development of synapses in both vertebrates and invertebrates. The *Drosophila melanogaster* neuromuscular junction (NMJ) is a glutamatergic synapse used as a model for the 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.

Though nearly twenty years have passed since the identification of the core BMP signaling components regulating synaptic growth and function, we still have an incomplete understanding of how the core pathway itself is regulated. 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. Loss of neuronal *Tao* results in overgrowth of synaptic termini without affecting muscle size, suggesting that Tao normally inhibits NMJ growth. Evoked release of neurotransmitter is also impaired in *Tao* loss-of-function, resulting in larval locomotion defects. 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, instead inhibiting the transcription of BMP target genes through a yet-unknown mechanism. We will present our recent progress made in identifying Tao's neuronal signaling partners and understanding the mechanism by which Tao and its partners inhibit BMP signaling during synapse development.

831B TRMT9B regulates synaptic function and motor behavior *Ambar Delgado*[1], Kimberly Rose Madhwani[2], Caley Hogan[3], Jennifer Dumouchel[4], Kate O'Connor-Giles[1,5] 1) Department of Neuroscience, Brown University, Providence, RI; 2) Neuroscience Graduate Training Program, Brown University, Providence, RI; 3) Laboratory of Genetics, University of Wisconsin-Madison, Madison, WI; 4) Therapeutic Sciences Graduate Training Program, Brown University, Providence, RI; 5) Carney Institute for Brain Science, Brown University, Providence, RI

The nervous system is highly dependent on the dynamic regulation of translation. Posttranscriptional modification of RNAs is emerging as a key regulator of protein synthesis. tRNAs are extensively post-transcriptionally modified to regulate their structure, stability, decoding speed, and accuracy. Through a genetic screen in Drosophila to identify new, conserved synaptic genes, we discovered that tRNA methyltransferase family member TRMT9B, also known as fid, negatively regulates synaptic growth. Here, we investigated TRMT9B's role in regulating neurotransmission under basal conditions and during homeostatic plasticity, and in locomotor behavior. Current clamp electrophysiology at the neuromuscular junction (NMJ) shows a significant decrease in neurotransmitter release in TRMT9B mutants. Considering the presence of ectopic synapses in TRMT9B mutants, this suggests diminished function of individual synapses at the NMJ and a role for TRMT9B in establishing precise synaptic communication.

Synapses modulate their strength during plasticity and to homeostatically maintain function at physiological levels in response to perturbation. At the Drosophila NMJ, disruption of postsynaptic glutamate receptors triggers a compensatory increase in presynaptic neurotransmitter release that precisely offsets the deficit to maintain optimal synaptic communication. Chronic presynaptic homeostatic potentiation (PHP) depends on the dynamic regulation of gene expression, so we investigated the role of TRMT9B in chronic PHP. Interestingly, we found that loss of glutamate receptor IIA resulted in lethality in the absence of TRMT9B. While this lethality precludes electrophysiological analysis, it suggests that long-term homeostatic compensation of neurotransmission may require TRMT9B. Finally, we investigated TRMT9B's role in locomotion through larval crawling and climbing assays, and observed significant impairment at both developmental stages. Overall, our findings indicate critical roles for TRMT9B in motor function.

832C Na+/H+ exchanger (Nhe) regulates neuronal morphology at the neuromuscular junction *Ashley Bielawski*[1], Isabella Maag[1], Beverly Piggott[1,2] 1) University of Montana, Missoula, MT; 2) Center for Biomolecular Structure and Dynamics, University of Montana, Missoula, MT

Emerging evidence points to a link between pH imbalances, and psychiatric conditions such as schizophrenia, autism spectrum disorder, and bipolar disorder. Due to its wide-ranging potential for new treatment approaches, it is imperative to establish whether altered pH may influence neural development and drive disease pathology. Intracellular pH can influence critical developmental pathways including cell migration, ion channel activity and abundance, and protein trafficking. However, it's not clear which of these processes act as pH-dependent modifiers of neuronal development. Na+/H+ exchangers (Nhe) are major regulators of pH and electrolyte balance, essential for brain development and neural

function. Disruption of Nhes in early life is associated with developmental disorders including microcephaly, intellectual disabilities, autism, and febrile seizures, but the specific role of Nhes in disease manifestation is not well understood. Compared to mammalian genomes which typically encode 9 Nhe proteins, *Drosophila melanogaster* encodes 3, making them a more straightforward model to elucidate Nhe function. Development of the *Drosophila* neuromuscular junction (NMJ) is an activity-dependent process, which precisely controls the number of synaptic boutons. It is known that pH-sensitive, presynaptic ion channels play a role in regulating neuronal excitability and bouton number at the NMJ. In addition, transient changes in pH at the synaptic cleft have been observed following an action potential, but how pH influences neuronal development and morphology is unclear. We find that altered Nhe2 expression affects bouton number at the NMJ. This work defines a new role for Nhe proteins in neuronal morphology and development.

833A Ion channel trafficking is coordinated with dendrite morphogenesis in sensory neurons Josephine Mitchell[1], *Ipek Midillioglu*[2], Jill Wildonger[2,4], Kathy Wang[3], Chun Han[3] 1) Biochemistry Department, University of Wisconsin-Madison, Madison, WI; 2) Pediatrics Department, University of California, San Diego, La Jolla, CA; 3) Weill Institute for Cell and Molecular Biology and Department of Molecular Biology and Genetics, Cornell University, Ithaca, NY; 4) Division of Biological Sciences, Cell & Developmental Biology Section, University of California, San Diego, La Jolla, CA

An organism's ability to perceive stimuli, such as pain, from its surrounding environment is essential to survival. The perception of an external stimuli and its transformation into a cellular response depends on ion channels that are distributed throughout the dendritic arbor of peripheral sensory neurons. However, it is not well understood how trafficking of ion channels to their sites of function in the dendritic membrane is regulated and whether or how their localization is coordinated with dendrite morphogenesis. We investigated how ion channel localization is regulated using the Drosophila melanogaster class IV dendritic arborization neurons. In larvae, painful mechanical stimuli are sensed in part by pickpocket 1 (ppk1) and pickpocket 26 (ppk26), which are members of the degenerin/epithelial Na+ channel/acid sensing ion channel (DEG/ENaC/ASIC) family. Ppk1 and ppk26 form a heterotrimeric channel in the class IV sensory dendrites and are dependent on each other for membrane insertion. To study the regulation of pickpocket ion channel trafficking, we used CRISPR-Cas9 genome engineering to fluorescently tag endogenous ppk1. We found that ppk1 localizes robustly and uniformly throughout the dendritic membrane and is present in actively growing dendrite tips, indicating that sensory function is established during dendrite morphogenesis. To delineate mechanisms that regulate ppk1 trafficking we investigated the endocytic network. We hypothesized that Rab11 may be involved in forward trafficking of ppk channels to the membrane and/or through local regulation via recycling pathways. We found that ppk1 and ppk26 localization to the dendritic membrane depends on the recycling endosome GTPase Rab11. Additionally, we found that ppk1 and ppk26 colocalize with the early endosome GTPase Rab5. When ppk1 is absent, ppk26 is not membrane expressed and instead forms puncta throughout the dendrites. Over half of these ppk26 puncta colocalize with Rab5, suggesting involvement of early endosomes in quality control of pickpocket channel trafficking. Together, our results suggest that pickpocket channel trafficking is coordinated with sensory dendrite morphogenesis and that its membrane expression relies on the endosomal network.

834B Na+/H+ Exchangers play essential roles in neurogenesis Ashley Bielawski[1], Isabella Maag[1], *Beverly Piggott*[1,2] 1) University of Montana, Missoula MT; 2) Center for Biomolecular Structure and Dynamics, University of Montana, Missoula, MT

Electrolyte balance and the maintenance of physiological pH are important in all cells across the animal kingdom. Sodium (Na+) Proton (H+) Exchangers (Nhe) are powerful proteins with the capacity to shape their cellular environment by acting as major modifiers of electrolyte balance and pH. They have emerged as important regulators of cell size, cellular metabolism, and neural excitability. Despite their potential to influence many key processes in brain development, their role in neurogenesis is not well defined. Human mutations in Nhe proteins are found to cause microcephaly, autism, and epileptic seizures, but how they drive disease pathology is not well understood. Defining the role of Nhe proteins in neurogenesis and disease is complicated by the fact that there are 9 Nhe proteins encoded by the human genome, which influence pH at the cell membrane as well as within intracellular organelles. As flies have only 3 Nhe proteins, we can use this simplified system to define precise defects in proliferation, neural formation and identity that are caused by loss or mutation of Nhe proteins. Our preliminary findings reveal that reduction/loss of Nhe proteins reduces overall brain size and influences proliferation in specific neuroblast lineages. This work will define pH-sensitive cellular processes and signaling pathways regulated by Nhe proteins.

835C Glia-dependent regulation of synapses in the *Drosophila* antennal lobe *Dan Jindal*[1], Isabella Bacon[1], Elizabeth Seitz[1], Sugapradha Saravanan[1], Alexis Bell[1], Rhea Mahajan[2], Heather Broihier[1] 1) Case Western Reserve University School of Medicine, Cleveland, OH; 2) Hathaway Brown School, Shaker Heights, OH

Developing nervous systems initially form with an overabundance of synapses. The conserved process by which excess synapses are eliminated to yield mature nervous system circuitry is known as synaptic pruning. Beyond traditional supportive roles in neuronal homeostasis and function, glia are the primary effectors of synaptic pruning and clear

synapses in part by phagocytosis. The mechanisms that tune glia-mediated synaptic pruning over development and adulthood are largely unclear.
Draper, an ortholog of Ced-1/MEGF10/Jedi, is the conserved surface receptor on glia that mediates engulfment and clearance in *Drosophila*. Glia require Draper to restrict presynapse number to normal levels in the adult antennal lobe. Directed screens are ongoing to reveal putative genes upstream of Draper that control the glial phagocytic program to allow for normal presynapse morphology. In tandem with this screen, it is important to assess (1) the effect of adult age on observed differences in presynapses between wild-type and candidate gene knockdown flies and (2) the split in pruning responsibility over time between two glial subtypes in direct contact with synaptic neuropil: astrocytes and ensheathing glia.
After this screen, it is crucial to characterize the effect of positive hits on the postsynaptic side of antennal lobe synapses. Beyond development, synaptic pruning is utilized by processes in the adult CNS like memory formation and maintenance. And so, the when and where of identified genes in regulating synapses is important; the developmental timing and the specific glia in which these genes act. Lastly, it is important to determine the functional impact of screened genes on adult olfaction and memory. Given that dysfunctional signaling between glia and synapses contribute to a host of synaptopathies, understanding the signaling pathways that direct the balance of synapse formation and removal is of significant therapeutic interest.

836A Exploring the role of glial Syndecan on neuroepithelium expansion in the *Drosophila* optic lobe *Duo Cheng*, Jaimy Coates , Vanessa Auld University of British Columbia

In order to establish a functional brain, neural stem cells (NSC) need to generate an abundant number of neurons and glia under tight temporal and spatial regulations. The *Drosophila melanogaster*'s optic lobe is a popular model to study neurogenesis as each optic lobe is generated by neuroblasts (NB) that differentiate from the columnar neuroepithelium (NE). Glia provide essential modulation to the NSC niche to ensure appropriate NSC differentiation. During the 3rd instar larval stage, the OPC+NE are surrounded by two distinct classes of glia, the subperineurial glia (SPG) and the cortex glia (CG). Both are known to modulate the NSC niche and play a role in NSC homeostasis. Yet the tools by which glia utilize to interpret and control the NSC niche are far from being fully characterized. Here, we explored the role of a transmembrane heparan sulfate proteoglycan (HSPG), Syndecan (Sdc) in regulating the NSC niche during development. Using super-resolution microscopy, we revealed Sdc is widely expressed across the brain lobe, though we notice Sdc is particularly enriched around the NE. Pan-glial knockdown of Sdc revealed a dramatic decrease in brain lobe volume, accompanied by a reduction of the NB population size within the OPC. Moreover, we observed the lengthening of the ventral nerve cord, indicative of disruption between glia and the overlying extracellular matrix that coats the entire nervous system. While the loss of Sdc in glia did not significantly alter the level of apoptosis, it did lead to a reduction in mitotic events in the OPC. Upon further experiments, we observed pan-glial Sdc knockdown perturbed the NE morphology, and reduced Notch activity within the NE. Notch signaling is known to prevent premature differentiation of NE into medulla neuroblasts, ensuring the expansion of the NE. Therefore we are investigating the role of Sdc in mediating Notch signaling cross-talk between the subperineurial glia and the NE. We are also exploring the potential influence of cortex glia on OPC proliferation and NE morphology. Overall, our results support a novel aspect of Sdc's role in regulating the NSC niche through glial regulation of the NE in the developing brain lobe.

837B Glia-derived lipid binding protein confers resistance to oxidative stress in the Drosophila brain *Jun Yin*, Hsueh-Ling Cheng, Jingce Lei, Anna Grigsby-Brown, Mary Gibbs, Aidan Dermady, Quan Yun National Institute of Neurological Disorders and Stroke, NIH, Bethesda, MD

Lipid trafficking between neurons and glia contributes to the development, function, and stress responses of the nervous system. Due to the diversity of lipid transport proteins and lipoprotein receptors, cellular and molecular pathways involved in neuron-glia lipid shuttling have not been well characterized *in vivo* under physiological conditions. How neurons obtain their lipid supply during development and how synaptic activity regulates this process remain open questions. To identify the molecular network involved in this process, we performed genetic screens on glia-derived secretory factors and evaluated their distributions and functions in the *Drosophila* central nervous system (CNS). One of our top hits is Odorant-Binding-Protein 44a (Obp44a), a small secretory protein highly expressed in both larval and adult astrocytes. Structure homology modeling indicates OBP44a as a lipid binding protein that potentially interacts with both fatty acids and cholesterol. Using CRISPR-medicated genome editing, we generated knock-in and knock-out OBP44a alleles, validated its glial expression and revealed its specific functions in regulating the physiological properties of fly neurons. Notably, OBP44a level is upregulated in flies challenged with the H2O2 treatment, while the mutant shows a significant reduction in their resistance to oxidative stress, as well as deficits in locomotor activity and sleep maintenance. Based on these findings, we propose that Obp44a functions as an antioxidant that protects lipids from peroxidation within the fly brain and represents a new class of glia-derived molecules contributing to the unique CNS lipid environment.

838C Divergent signaling requirements of dSARM in injury-induced degeneration and developmental glial phagocytosis *Yizhou Liu*[1], Kelsey Herrmann[1], Arnau Llobet-Rosell[2], Colleen McLaughlin[1], Lukas Neukomm[2], Jaeda

Coutinho-Budd[3], Heather Broihier[1] 1) Department of Neuroscience, Case Western Reserve University School of Medicine, Cleveland, OH; 2) Department of Fundamental Neurosciences, University of Lausanne, Lausanne, Switzerland; 3) Department of Biology, University of Vermont, Burlington, VT

Elucidating signal transduction mechanisms of innate immune pathways is essential to defining how they elicit distinct cellular responses. Toll-like receptors (TLR) signal through cytoplasmic TIR domains that bind other TIR domain-containing adaptors. dSARM/SARM1 is one such TIR domain adaptor best known for its role as the central axon degeneration trigger after injury. In degeneration, SARM1's domains have been assigned unique functions: the ARM domain is auto-inhibitory, SAM-SAM domain interactions mediate multimerization, and the TIR domain has intrinsic NAD^+ hydrolase activity that precipitates axonal demise. Whether and how these distinct functions contribute to TLR signaling is unknown. Here we show divergent signaling requirements for dSARM in injury-induced axon degeneration and TLR-mediated developmental glial phagocytosis through analysis of new knock-in domain and point mutations. We demonstrate intragenic complementation between reciprocal pairs of domain mutants during development, providing evidence for separability of dSARM functional domains in TLR signaling. Surprisingly, dSARM's NAD^+ hydrolase activity is strictly required for both degenerative and developmental signaling, demonstrating that TLR signal transduction requires dSARM's enzymatic activity. In contrast, while SAM domain-mediated dSARM multimerization is important for axon degeneration, it is dispensable for TLR signaling. Finally, we find that dSARM functions with the MAP3K Ask1 during development but not in degenerating axons. Thus, we propose that dSARM exists in distinct signaling states in developmental and pathological contexts.

839A Characterising the molecular basis of Drosophila glial diversity *Inês Lago-Baldaia*[1], Austin Seroka[2], Chintan Trivedi[1], Maia Cooper[1], Gareth Powell[1], Stephen Wilson[1], Sarah Ackerman[2], Vilaiwan Fernandes[1] 1) Department of Cell and Developmental Biology, University College London, London, UK; 2) Institute of Neuroscience, Howard Hughes Medical Institute, University of Oregon, Eugene, OR, USA

Glia form a vital part of the nervous system and play pivotal roles in nervous system development and function. Nonetheless, they have been understudied and are poorly characterised. In Drosophila, glia are broadly classed as surface glia, cortex glia, ensheathing glia and astrocyte-like glia. Whether these categories can be further subdivided to define unique cell types is unclear. Recent advances in single cell transcriptomics have been invaluable for rapidly characterising and defining cell types in molecular terms. Here we used published single cell RNA sequencing (scRNA seq) datasets of the complex but spatially ordered Drosophila optic lobe to characterise glial diversity in molecular terms. The optic lobe contains ~14 morphologically distinct glia, making it an ideal system to study glial diversity. We first used the R *Seurat* package to integrate two independent glial scRNA seq datasets (Özel *et al*., 2020; Kurmangaliyev *et al*., 2020) for the adult optic lobe. This analysis generated 17 distinct clusters. We used *in situ* hybridisation chain reaction (HCR) and reporter lines to test *in vivo* expression of cluster markers and annotated 15 of the clusters. We determined that the majority of specialisation exists between the lamina neuropil and the rest of the optic lobe, with lamina-specific subtypes of cortex, astrocyte-like and ensheathing glia. While our bioinformatic analysis of non-lamina glia generated multiple clusters with either astrocyte-like, ensheathing or cortex glial molecular characteristics, these could not be explained by morphological or regional differences when we validated markers *in vivo.* We speculate that at least some of these clusters may correspond to distinct cell 'states' rather than 'types'. On the other hand, our analysis also uncovered unexpected diversity within the chiasm glia. We have used annotated glial clusters from the adult dataset to identify glia from earlier developmental stages beginning with the third larval instar (Konstantinides *et al*., 2021). We have also compared glial transcriptomes of the embryonic central nervous system (unpublished) and adult optic lobe and found remarkable overlap in gene expression, suggesting a very strong maintenance of cell identity. Thus, by creating a Drosophila glial atlas, our work has begun to provide important insights into glial diversity and development.

840B Regulation of Glial Septate Junction proteins by microRNA-184 *Sravya Paluri*, Vanessa Auld Life Sciences Institute, University of British Columbia, Vancouver, Canada

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 (k-k) and sinuous (sin). Barriers formed at the convergence of three SJ form the tricellular junction (TCJ) which include 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 distribution and regulation in the nervous system is less studied. microRNA-184 (miR-184) is predicted to target a wide range of SJ and TCJ mRNAs. miR-184 targeting SJ proteins in epithelia was confirmed, however its role in nervous system is still unknown. While the presence of NrxIV is well established in glial SJ, we tested for distribution other SJ miR-184 targets within glia and established the presence of kune-kune, Mcr and sinous in glial SJs, and M6 and Gli in glial TCJs. miR-184 overexpression in SPG led to complete SJ and TCJ degradation and loss of these proteins from the membrane. Moreover, these larvae exhibited pupal lethality

and decreased larval locomotion. In future, we will assess BBB integrity by dye penetration assays to understand the functional implication of SJ degradation. To determine which SJ mRNAs are affected by miR-184, we will quantify mRNA levels for each potential target using qRT-PCR. These results will reveal if miR-184 directly targets all SJ mRNAs predicted to be targets of miR-184, or just key SJ mRNAs which affect other SJ protein localization. A potential candidate for the latter possibility is NrxIV, which is a core protein that mediates SJ assembly. We will analyze the role played by NrxIV by testing if NrxIV mRNA lacking the miR-184 target sequence can rescue the SJ degradation phenotype. Our study will reveal the presence and regulation of key SJ proteins in SPG crucial for BBB maintenance. Degradation of permeability barriers in glia can result in a compromised BBB—a hallmark of many diseases across many species.

841C Investigating the localization and function of laminin and dystroglycan in *Drosophila* wrapping glia development *Katherine Clayworth*, Vanessa Auld Life Sciences Institute, University of British Columbia, Vancouver, BC

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, suggesting 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). These results potentially indicate a form of WG polarization, a feature that has 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. Therefore, we are in the process of tagging alternatively spliced exons of Dg, which will allow us to follow their localization *in vivo*. 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.

842A Identifying subperineurial glia-specific dlg1 isoforms required for septate junction function *Mary Gilbert*, Vanessa Auld University of British Columbia

Septate junctions (SJ) within the subperineurial glia (SPG) form permeability barriers in Drosophila that protect central and peripheral neurons from exposure to the hemolymph; the blood-brain and blood-nerve barriers. The ladder-like structure (or septa) of SJs forms “rails” running parallel to two adjacent cell membranes between neighboring cells or autotypically in the same cell along the length of the glia, and these create the physical permeability barrier. Core components of SJs (such as NeurexinIV, Neuroglian and the Na/K ATPase pump) are defined as proteins that create the physical junction and are essential for SJ integrity, where the absence of any one leads to a compromised permeability barrier. Another critical component of the SJ is discs large (dlg1), a polarity protein necessary for SJ scaffolding. Though how dlg1 functions to ensure SJ development and maturation is not known. Dlg1 plays wide range of cellular roles including neuromuscular junction formation, establishing polarity in epithelial cells and neuroblasts, and scaffolding various protein complexes to the cytoskeleton. The *dlg1* gene organization is complex with two start sites and alternatively spliced exons capable of expressing at least 21 transcripts with a variety of functional domains including S97, 3 PDZs, SH3 and GUK domains. I have found that existing loss of function dlg1 alleles or RNAi-mediated knockdown disrupts the morphology and integrity of the glial SJs. However it is unclear which *dlg1* isoforms and which domains are required in glial SJ formation. I am using a two-part strategy to investigate the which *dlg1* isoforms are present in SPG and which *dlg1* isoforms are necessary for SPG SJ function. I am using RNAseq approaches to identify dlg1 transcripts from isolated and purified SPG mRNA. I am using CRISPR based approaches to generate complete deletion alleles that remove the two transcriptional start sites (S97 start site or the dlgA start site). I plan to test the requirement of each SPG dlg1 isoform by expressing dlg1 transgenes in these null backgrounds to determine which subset is able to rescue the SJ defects. In this way I will determine which domains of dlg1 are necessary for the formation of the Drosophila blood-nerve and blood-brain barriers.

843B Exploring molecular mechanisms of *Abnormal spindle* function in brain growth and development *Shalini Chakraborty*, Todd Schoborg University of Wyoming

Autosomal recessive primary microcephaly (MCPH) is a congenital condition which is characterized by smaller brain size,

intellectual disabilities, and life span reduction. Human MCPH is most commonly caused by homozygous mutations in the *abnormal spindle like microcephaly associated* (*aspm*) gene, which has a *Drosophila* ortholog called *abnormal spindle (asp).* Recent structure-function studies from our lab have identified a ~600 amino acid 'minimal fragment' (Asp^{MF}) of Asp's N-terminus that is sufficient to rescue brain size in *asp* mutant flies. Although the MCPH phenotype is clinically well-characterized, the cellular basis of this disorder remains unknown. As an approach to uncover the cellular and molecular basis of MCPH, we employed a high-definition yeast two hybrid (Y2H) screen to identify potential interactors of Asp^{MF}. Using a third-instar larval brain cDNA library, we screened over 100 million potential domain-level interactions with Asp^{MF}. Gene ontology analysis of the candidate interactors revealed molecular functions including microtubule and actin cytoskeleton related functions in addition to signaling pathways involved in cell differentiation. Genetic rescue experiments suggest that these interactions are specifically required in neuroepithelial and lamina precursor cells of the larval brain during the neurogenic time window of development in order to promote proper brain size and morphology. We will discuss these ongoing studies to identify the molecular basis of MCPH and Asp's ability to promote proper brain growth and development

844C The neurodevelopmental transcriptional landscape of a fly model for human microcephaly *Constanza Mannino*, Mercedes Bartels-Cassidy, Todd Schoborg University of Wyoming

Autosomal recessive primary microcephaly (MCPH) is a congenital disease characterized by reduced brain size which affects 2-12 cases per 10,000 live births in U.S. There are currently 27 genes related to the MCPH phenotype. Mutations in the Abnormal Spindle-like, Microcephaly Associated (ASPM) gene account for the majority of human MCPH cases; this gene has an ortholog in Drosophila, abnormal spindle (asp), that displays the same small brain phenotype. Although mitotic spindle defects have been proposed to be the major driver of asp mutant brains, previous work from our lab and others have shown that the etiology of MCPH is complex and that multiple cellular pathways likely contribute to this disorder. This likely reflects additional roles that Asp has during neurogenesis. To uncover these roles and identify new pathways involved in MCPH, we analyzed the transcriptional landscape of asp mutant brains across three key neurodevelopmental time points: neurogenesis (3rd instar larvae), neuronal tissue remodeling (mid-pupae), and neural homeostasis (adult). Differential expression analysis revealed that the most significant expression changes (>1,000 genes, F.C. >0.5, P-value <0.05) were observed during the neurogenic and homeostasis periods, suggesting that neural remodeling does not play a major role in shaping the asp MCPH phenotype. GO analysis revealed a number of enriched terms, including an upregulation of immune system (NF-KB, Toll) and stress-related MAPK pathways that were consistent between neurogenic and homeostasis stages. To functionally test whether these pathways contribute to the etiology of asp MCPH, we probed genetic interactions between asp and components of the NF-KB (rel) & Toll (dif) pathways. Brain size was partially restored in asp/rel and asp/dif double mutant animals, despite no rescue of the neuropil disorganization phenotype. These results suggest that the inflammatory response may partially contribute to the asp small brain phenotype through a growth control mechanism that is currently under investigation. We will also present ongoing studies functionally testing the importance of MAPK-stress related pathways in the etiology of asp MCPH.

845A Innate immune signaling sculpts neuron-glia interactions across lifespan Heather Broihier Case Western Reserve University

Developmental neurobiologists have been consumed with elucidating how circuits are built and remodeled since the 1940s when Roger Sperry formulated the chemoaffinity hypothesis. Their work has led to key insights into molecular mechanisms responsible for driving axon guidance, target recognition, and synaptogenesis. What is much less clear is how these generative processes are balanced with degenerative processes. Neurons that are damaged, those with aberrant electrical activity, or lacking trophic support often undergo apoptosis during development and in the adult. And at a smaller scale, pruning of neurites and synapses is key to circuit remodeling and plasticity.
Toll-like receptor (TLR) pathways were first identified for their roles in embryonic patterning in flies and have since been defined as a conserved centerpiece of innate immunity. My lab elucidated functions of TLR signaling in neural development, specifically at the NMJ. We then made the unexpected discovery that one of the most pronounced phenotypes associated with loss of Toll-6 function, a dramatic increase in the number of apoptotic neurons during development, is caused by selective loss of Toll-6 function not in neurons, but rather in glia. We demonstrated that release of the Toll-6 ligand, Spz5, from dying neurons activates a novel Toll-6 pathway in glia to prime them for phagocytosis. I will discuss recent progress my laboratory has made defining the functions of TLR signaling in regulating neuron-glia interactions in synapse, neurite, and neuron elimination across lifespan.

846B Response to and regulation of codon bias in *Drosophila* neural lineages. *Rebeccah Stewart*, Scott Allen, Don Fox Duke University

Codon bias occurs when certain codons appear more frequently in the coding genome than their synonymous counterparts. This phenomenon occurs throughout all forms of life and is important in determining protein expression at the steps of both transcription and translation. Although the impact of codon bias is only beginning to be studied during animal development and in different tissues, an understanding of how individual cell types respond to codon

biased transcripts is beginning to emerge. We have undertaken one of the first studies to reveal cell and tissue-specific differences in codon bias in the model organism *Drosophila* and have uncovered some of the first evidence that tissues in the same animal respond differently to codon usage *in vivo*. Specifically, in cell types within the developing *Drosophila* brain, we find robust protein expression from reporters enriched in rarely used codons, whereas most other tissues in the fly do not express such reporters. Within the brain itself, neural stem cells are unable to express proteins encoded by rare-codon enriched genes, while differentiated neurons can. Cell identity appears tied to the ability to express transcripts enriched in rare codons, as experimentally blocking neuronal differentiation drastically reduces brain expression of a rare-codon enriched reporter. To uncover molecular regulators that enable neurons to specifically express rare-codon enriched reporters, we have initiated a reverse genetic screen. This screen used RNAi and the UAS-GAL4 system to knock down potential regulators enriched in neuroblasts or neurons. We assessed the role of each gene in enabling translation of rare-codon enriched transcripts by measuring expression of our rare-codon enriched reporter in neurons. Results of the screen will be presented at the poster. Using our *Drosophila* model, we may ultimately identify a new regulatory mechanism during neuronal development and function: cellular response to codon bias.

847C Charting the development of leg sensory organs at the single-cell level *Ben Hopkins*, Olga Barmina, Tiezheng Fan, John Larue, Artyom Kopp University of California – Davis, Davis, CA

To respond to the world around them, adult flies rely on the input of a network of diverse sensory organs that are distributed across the body. The developmental genetic processes that drive divergence between different classes of sensory organs remain poorly resolved. Yet resolving these processes is critical to understanding how sensory systems function and evolve. Progress in this area has been limited by the difficulty of characterizing transcriptomic differences between sensory organs using bulk and single-cell RNA-seq (scRNA-seq): many sensory organs are rare, patchily distributed, difficult to dissociate into constituent cell-types, and embedded in undigestible cuticle. To overcome these difficulties, we developed a fine-scale dissection technique and dissociation protocol to target the pupal first tarsal segment of *D. melanogaster* males for scRNA-seq at multiple time points. This region is uncommonly enriched for sensory organs, containing large numbers of mechanosensory and chemosensory bristles, campaniform sensilla, the distal tibial chordotonal organ, and the sex comb, a male-specific evolutionary innovation present in a subset of Drosophilid species. Our datasets have allowed us to resolve distinct expression profiles for constituent bristle cells (sockets, shafts, sheaths, and neurons) separately for mechanosensory and chemosensory bristles and sex comb teeth. Furthermore, by subclustering neuronal populations, our data point to a combinatorial transcription factor code that specifies each of the four different chemosensory neuron classes (including male- and female-pheromone sensing neurons) and mechanosensory neurons. We verify this code using a combination of transgenic reporters and antibody staining. Finally, we recover a small population of neurons, enriched for the Pax family transcription factors *eyg* and *toe*, that mark campaniform sensilla. Using overexpression and RNAi experiments, we show that the expression of *eyg* and *toe* in campaniform sensilla suppresses bristle growth, giving rise to the characteristic dome morphology of these strain-detecting organs. Collectively, our work describes an array of developmental genetic differences that define the diverse sensory capabilities of the adult leg.

848A Uncovering the mechanism of *slit* function in PNS development *Maria Alejandra Pizarro Salazar*, Afshan Ismat University of St. Thomas

Slit is a secreted ligand expressed in the ectoderm and plays an important role during neurogenesis. In the peripheral nervous system (PNS), removing *slit* results in defects in the morphology and the migration of the lch5 chordotonal neurons. In order to gain a deeper understanding of how *slit* functions in this developmental process, we have over-expressed *slit* in the neurons, glial cells, or ectoderm of Drosophila embryos, and examined PNS neurons in each situation. Preliminary results show that over-expression of *slit* in these tissues results in a range of defects in comparison to wild type embryos. Over-expression of *slit* in glial cells results in the loss of certain dorsal neurons. The over-expression of *slit* in neurons shows that the cluster tails from the dorsal section are shorter than those found in wild type embryos. Additionally, over-expression of *slit* in the ectoderm results in defects including an axonal branch from one section that connects to another and shows a neuron that is completely out of place. The out-of-place neuron appears mostly close to the dorsal tail of sections A2-A3 with the direction of the axon going towards the ectoderm. These results suggest that Slit could be working as an attractant instead of a repellent for the neurons of the PNS. Currently, we are in the process of testing this hypothesis further. Clearly, this data demonstrates the importance of *slit* in PNS development, and we hope to provide insight into the mechanism of *slit* function in the PNS.

849B Delta/Notch signaling inhibits expression of the early temporal factor Imp to promote termination of neurogenesis during development *Chhavi Sood*, Sarah Siegrist University of Virginia

In most metazoans, neural stem cells (NSCs) proliferate throughout development and then terminate their cell divisions prior to developmental completion. This ensures the formation of an adult neural circuit system that is functional and stable. To better understand the cellular and molecular mechanisms that regulate termination of NSC cell divisions and neurogenesis during development, we carried out a large scale GAL4/UAS-RNAi screen in *Drosophila*. From this screen,

we identified the Notch receptor and its ligand Delta. When Notch or Delta are knocked down, neuroblast divisions in the central brain region continue into adulthood, whereas when Notch is constitutively activated, neuroblast divisions terminate prematurely. We found that defects in Notch-mediated timing of termination are due to defects in neuroblast temporal patterning. When either Notch or Delta are knocked down, expression of the early temporal factor Imp (IgF-II mRNA binding protein) is maintained longer compared to controls, concomitant with delayed expression of the late acting temporal factor, Syp (Syncrip). This leads to an increase in the number of Imp and Syp double positive neuroblasts, or neuroblasts with mixed temporal identity and reduced expression of the even later temporal factor, E93 (Eip93F). However, surprisingly, when we knocked down Imp and Notch signaling or overexpressed Syp and knocked down Notch signaling, the central brain neuroblasts still persisted into adulthood. This suggests that Delta/Notch cell signaling may regulate both temporal patterning and neuroblast cell cycle exit. To better understand whether Notch regulates neuroblast cell cycle exit independent of Imp/Syp temporal patterning, we assayed neuroblast quiescence during the embryonic to larval transition. When Notch is knocked down, some neuroblasts fail to enter quiescence and continue proliferating during the embryonic to larval transition. We conclude that the evolutionarily conserved Delta/Notch cell-cell signaling pathway regulates the Imp to Syp temporal transition and regulates neuroblast cell cycle exit. While Notch signaling is well known for its role in regulating binary cell fate decisions, it is becoming more apparent that Notch signaling also plays important roles in binary temporal decisions, in this case, early versus late.

850C Deciphering the molecular clock controlling the neurogenesis diversity in drosophila's medulla *Khaled Ben el kadhi*[1], Claude Desplan[1,2] 1) New York University Abu Dhabi, Abu Dhabi, UAE; 2) New York University, NY, USA

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 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.
Obtaining these dynamic data will allow us to decipher the molecular clock of tTFs 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.

851A Exploring the Role of Retrotransposable Elements in the Development of Microcephaly Bert Crawford[1], Sabrina Torres[1], *Michelle Longworth*[1,2] 1) Cleveland Clinic Lerner Research Institute; 2) Cleveland Clinic Lerner College of Medicine of Case Western Reserve University

Microcephaly is a rare disorder in which babies are born with head sizes that are approximately three standard deviations below the mean. Mutations in twenty-five proteins (termed microcephaly proteins), including three condensin subunits, have been shown to cause microcephaly in humans and in mouse models. However, the mechanisms by which microcephaly protein depletion and/or dysregulation leads to the development of microcephaly are not well understood. Condensins are conserved, multi-subunit complexes that are important for regulating chromosome organization. Our lab discovered that condensins also repress the expression and movement of retrotransposons in both *Drosophila melanogaster* and in human cells.

To determine whether increased retrotransposon expression and activity might contribute to the development of condensin-deficient microcephaly, we first used the UAS-GAL4 system to deplete condensin proteins in specific cell types of the developing *Drosophila* brain. Depletion of various condensin proteins in stem cells (neuroblasts), but not glial cells or post-mitotic cells resulted in significantly smaller adult brains and adult heads. We observed increased transcript levels of several families of retrotransposons and endogenous retroviruses (ERVs) in larval brains deficient for the condensin II subunit dCAP-D3. Experiments using gypsy-CLEVR reporter stocks demonstrated that replication of the gypsy retrotransposable element/ ERV is prevalent in wild-type larval neuroblasts, and is significantly increased in

dCAP-D3-deficient neuroblasts. Further, dCAP-D3-deficient larval neuroblasts exhibited increased lagging chromosomes, a phenotype observed in microcephaly patients harboring condensin mutations. dCAP-D3-deficient pupal brains also exhibit increased cell death. Excitingly, condensin-deficient microcephaly was almost completely rescued by allowing flies to develop on food containing Nucleoside Reverse Transcriptase Inhibitors (NRTIs), which inhibit retrotransposition and ERV replication. Together, these findings suggest that condensins may repress retrotransposon and ERV expression and activity in the developing *Drosophila* brain to prevent microcephaly. We will also present data which suggests that other microcephaly proteins may be involved in these processes.

852B Long-range temporal patterning of progenitors in the developing *Drosophila* optic lobe *Ishrat Maliha Islam*[1,2], Urfa Arain [1,2], Priscilla Valentino [1,2], Ted Erclik [1,2] 1) Department of Cell and Systems Biology, University of Toronto, Toronto, ON; 2) Department of Biology, University of Toronto (Mississauga), Mississauga, ON

The *Drosophila* optic lobe serves as an excellent model system in which to study the mechanisms that regulate neurogenesis. The largest neuropil of the optic lobe, the medulla, is comprised of 40 000 neurons belonging to over 90 neuronal types. These neurons are generated from a neuroepithelial crescent called the outer proliferation center (OPC). At the beginning of the 3rd larval instar, a proneural wave converts each neuroepithelial cell into a neuroblast (NB), which subsequently divides asymmetrically to generate two neuronal or glial cells. It has been previously shown that OPC NBs generate unique sets of neurons based on their spatial origin and temporal state. In the spatial axis, the OPC is subdivided by the expression of Vsx1, Optix, Rx and Hh to generate eight distinct compartments. In the temporal axis, each NB sequentially expresses a cascade of five temporal transcription factors (tTFs) - Hth, Ey, Slp1/2, D and Tll - as it ages. Therefore, as the NBs in each OPC spatial domain divide, they rapidly cycle through the tTFs to generate a set of unique neurons at each spatio-temporal birth address. Here, we describe a third patterning axis that further diversifies medulla neuronal fates. We show that the OPC neuroepithelium is patterned by the expression of five long-range temporal factors; Imp, Syp, Chinmo, Mamo and E93. Over the course of the two days of OPC neurogenesis, we find that Imp and Chinmo are expressed in descending gradients in the OPC neuroepithelium, whereas Syp is reciprocally expressed in an ascending gradient. We show that these genes are required for the expression of the transcription factors Mamo and E93 in early and late temporal windows, respectively. To determine whether these long-range temporal patterning genes are required for neuronal fate specification, we focused on the neurons that are generated from the Vsx1-Hth spatio-temporal window. Surprisingly, we find that four (not one) neuronal types – Tm23, TmY15, Pm3 and TmY12 - are sequentially generated by Vsx1+Hth+ NBs during the two days of neurogenesis. We further show that the long-range temporal genes are required for this unexpected diversity; *mamo* promotes early neuronal fates (Tm23 and TmY15), whereas *syp* is required for late fates (Pm3 and TmY12). Taken together, our findings show that medulla NBs integrate three inputs – one spatial and two concurrent temporal (rapidly changing tTFs and long-range) – to generate the extensive neuronal diversity observed in the medulla.

853C Intrinsic and Extrinsic Cues Regulate the Early-to-Late Transition of Transcription Factors in Drosophila Type II Neuroblast *Gonzalo Morales*, Mubarak Syed University of New Mexico

The central nervous system of D. melanogaster is composed of numerous morphologically different neurons and glia which originate from a limited number of type II neural stem cells (NSC). Such neural diversity poses the important question of how these few NSCs give rise to a variety of neurons and glia; furthermore, NSCs' failure to diversify in the brain results in severe brain pathologies and behavioral defects. D. melanogaster is a widely used model to test neuronal differentiation mechanisms and transcription-mediated development of neural circuits. Type II (TII) NSCs divide asymmetrically beginning in embryogenesis and continue to divide into late larval stages. Upon division, TII NSCs self-renew and produce an intermediate neural progenitor (INP) which will also divide asymmetrically into multiple progenitors that eventually compose most of the adult's central complex.
Recently, TII NSCs were found to express transcription factors in a temporal manner to produce neuronal diversity. Among these temporal factors, the ecdysone receptor (EcR) is essential because it initiates the early-to-late transcription factor transition, and its production takes place 56 hours after larvae hatch (ALH). Seven-up (svp) activity is required from 0-36 hours ALH for induction of EcR transcription, but svp is downregulated at 36 hours; therefore, the 20 hour time gap between svp disappearance and EcR appearance suggests an additional regulatory mechanism is responsible for EcR induction and NSC temporal cascade transition from the early to late stage in larvae. Previous studies have shown these molecular transitions are linked to intrinsic and extrinsic cues which play important roles in the larval NSC transcription factor cascade. Cell division is an internal process essential for neuroblast development, and may trigger an intrinsic process via the cell cycle to establish neural diversity and activate transitions in the temporal cascade. We hypothesize that if the cell cycle is blocked, then transcription factors inducing later-stage development of neuroblasts will be absent while early-stage transcription factors will continue to be expressed into L2/L3 stages. To test this, two kinds of cell cycle disruptors are used: first, a cytokinesis cycle delay signal (CDK-1RNAi) which inhibits the formation of a protein kinase subunit controlling important aspects of cell cycle progression; and second, pavarotti downregulation (PavRNAi) which inhibits transcription of microtubule motor proteins in spindle apparatus formation. Cell cycle disruption at both 0 and 48 hours ALH, which are critical time points for proper EcR induction, showed lack of EcR in the NSC at 56 hours. These

results support our hypothesis in that cell cycle disruption leads to persistent early-stage transcription factor expression into late larval developmental.

854A Unraveling the mechanisms of early neurogenesis with single cell resolution Ana Costa Veloso[1,2], *Robert Zinzen*[1,2] 1) Max Delbrueck Centre for Molecular Medicine (MDC), Berlin, Germany; 2) Berlin Institute for Medical Systems Biology (BIMSB), Berlin, Germany

The *Drosophila* embryonic nervous system develops from neuroblast stem cells that emerge in a characteristic spatiotemporal pattern per segment. The lineage relationships from neuroblast to neurons and glia are highly stereotypic and have largely been described, but the underlying mechanisms driving diversification, specification and differentiation are still poorly understood.
To uncover how neuroblasts produce distinct lineage trajectories, we have produced a gene expression atlas resolving over 65 000 neurogenic stem cells across 6 consecutive timepoints encompassing all waves of neuroblast delamination. The resulting transcriptomic blueprint of neuroblasts and ganglion mother cells allows unique and novel insights into the molecular mechanisms responsible for early nervous system development.
We have been able to identify specific neurogenic populations and their gene expression dynamics along early neurogenesis. Our resource allows assigning spatial and temporal identities to individual cells and discovery of new spatio-temporal markers, including transcription factors and signaling molecules. Moreover, we show that the gene expression programs of individual neuroblast identities can be linked to specific upstream regulators and give new insights into the downstream effectors of cellular behavior. Several neuroblasts with glial potential were particularly interesting, as their glial character becomes manifest not only much earlier than expected, but temporal ordering indicates 'developmental pockets' of signaling receptivity. Finally, we demonstrate that newly identified drivers of neurogenesis are functionally important for crucial features of neurogenesis, such as proper motoneuron projection. We present a resource that will aid in identifying neurogenic populations and individual neuroblasts, further our understanding of how individual neuroblasts diverge molecularly, allow discerning how their expression programs are regulated and how these differences drive the emergence of specific cell identities such as distinct motoneurons, interneurons, and glia.

855B Building an integrative model of how nutrition and natural genetic variation interact during neurogenesis in natural populations of *Drosophila melanogaster* *Taylor L. Nystrom*, Sarah Siegrist, Alan Bergland University of Virginia

During early life neurogenesis, neural stem cells give rise to thousands of morphologically and functionally diverse neurons that will build the adult brain. Ultimately, these neurons form functional circuits, and it is these circuits that allow proper functioning and survival of the adult animal. While neurogenesis is known to be influenced by both intrinsic and extrinsic signalling, we still have a limited understanding of how these signals interact. My work seeks to elucidate the integration of intrinsic and extrinsic signalling by studying how an extrinsic factor, nutrient signalling, and an intrinsic factor, natural genetic variation, interact during nutrient-dependent processes of neurogenesis in *Drosophila melanogaster* (fly). In order to capture genetic variation reflective of natural populations, I have used a collection of geographically diverse, wild-caught, inbred lines of flies for all experiments. I first characterized neural stem cell, or neuroblast (NB), reactivation, a temporally regulated, nutrient-dependent process of neurogenesis. My work revealed significant differences in the timing of NB reactivation from quiescence when genetically distinct flies are raised in a calorically rich environment. These differences in reactivation could affect the total amount of time that a NB is producing neurons, which could result in changes in the total number of neurons in the adult brain and potentially cause functional differences for the adult fly. Moreover, these results may suggest a genetically encoded basis for variation in neurogenesis.

After observing variation in neurogenesis in a calorically rich environment, I characterized NB reactivation in a calorically poor environment. Preliminary results show that flies with distinct genetic backgrounds respond differently to caloric restriction. Such results may suggest a gene-by-environment interaction during the transition from quiescence to reactivation in flies.

Next, I will continue to build a full timeline from reactivation to termination in larval NBs in calorie rich and calorie poor environments, measure temporally regulated transitions between transcription factors that play a role in neuronal specification, analyze the genomes of the experimental fly lines for genes involved in nutrient dependent steps of neurogenesis, and quantify the neuronal make-up of the adult fly brains. Through this work, I will build a more integrative understanding of how intrinsic and extrinsic cues interact during neurogenesis.

856C Establishing anterior-posterior diversity in how stem cells give rise to neural circuits for somatosensory processing *Deeptha Vasudevan*, Yi-Wen Wang, Hannah Carr, Sean Corcoran, Chris Wreden, Ellie Heckscher Department of Molecular Genetics and Cell Biology, The University of Chicago

In different anterior-posterior (A-P) locations of the body, there are different circuits that control specialized functions.

For example, humans have appendages (arms that are anterior, legs posterior to the trunk) that have unique movement patterns. Circuits at different A-P locations must process different sensory stimuli to generate specific movements. Spinal cord injury at different A-P levels gives different phenotypes. Injury in more anterior regions leads to paralysis of all 4 limbs whereas more posterior injuries result in paralysis of lower limbs, and regenerative treatments would need to generate region-specific circuits. The vertebrate spinal cord is segmented and develops from stem cells. In each segment, there are ~10 different progenitor domains that give rise to specific sets of spinal neurons. These 10 progenitor domains are repeated in every segment along the A-P axis. Stem cells have enormous therapeutic potential to regenerate circuits, but first we must understand how stem cells give rise to the A-P diversity of circuits during development. What are circuit variations in the spinal/ nerve cord along the A-P axis? And how are these circuit variations established in development? I am developing the *Drosophila* larval nerve cord as a model to address this question. Similar to the progenitor domains in the vertebrate spinal cord, the Drosophila nerve cord has a repeating set of ~30 stem cells called neuroblasts, which differentiate into neurons that wire up to form circuits. Here, I am characterizing the A-P circuit diversity of one neural stem cell lineage called neuroblast 3-3 (NB3-3). These NB3-3 stem cells are found in every segment of the *Drosophila* nerve cord and give rise to sensory processing even-skipped lateral (EL) interneurons. We are testing which properties of EL circuits are position-dependent vs position-independent. First, we assayed how ELs vary across the A-P axis of the Drosophila nerve cord in number, birth timing, and morphology using molecular and temporal identity markers, and stochastic labeling techniques. We are investigating diversity in sensory encoding of ELs by determining anatomical and functional input synaptic partnerships using transsynaptic circuit tracing and calcium imaging. This will tell us the diversity of somatosensory processing circuits in the nerve cord, as well as the diversity in how stem cells give rise to these circuits along the A-P axis. In the future, we can test genetic and cellular mechanisms that establish this A-P variation in circuit development.

857A The OTUD6 deubiquitinase associates with the 40S ribosome to regulate translation and the response to stressors in Drosophila *Sammy Villa*, Anika Padala, Frederick Wolf University of California, Merced

Ribosome ubiquitylation is a highly regulated process that is essential for maintaining proper protein translation. While much is known about the role of ubiquitin in these pathways, less is known about the function of deubiquitinases. OTUD6 is an ovarian tumor (OTU) family deubiquitinase that is conserved from yeast (OTU2) to humans (OTUD6A & B). To study OTUD6 in Drosophila, CRISPR/Cas9 was used to create epitope-tagged wild-type and catalytically dead (DUB-dead) endogenous OTUD6. DUB-dead and loss-of-function OTUD6 mutants are markedly sensitive to oxidizing (paraquat) and alkylating (mms) agents that impact protein translation and ribosome function. Co-immunoprecipitation coupled with mass spectrometry in OTUD6.DUB-dead brains, where OTUD6 is highly expressed, identified 40S ribosome and RNA exosome proteins. The most significantly enriched 40S proteins, RACK1 and RPS3, play key roles in ribosome quality control and turnover under cellular stress. Mutation of RACK1 rescued the mms sensitivity of OTUD6 mutants, indicating that OTUD6 is likely a negative regulator of RACK1. RACK1 regulates ubiquitylation of several 40S ribosome subunits: a genetic screen of E3 ligases that ubiquitylate the 40S ribosome in a RACK1 dependent manner revealed that OTUD6.DUB-dead interacts epistatically with two E3 ligases. Both E3 ligases are implicated in RpS3 ubiquitylation and in ribosome quality control. In support of OTUD6 regulating ribosome quality control or turnover, OTUD6 mutants have decreased protein translation, delayed development, and dramatically extended lifespan. Thus, we hypothesize that OTUD6 regulates protein translation through a novel deubiquitylation event on the 40S ribosomal subunit in response to stress conditions.

858B Rasputin – A mediator of translational activation for essential proteins in neurodevelopment *Al Rohet Hossain*, Kaicheng Ma, Kayla Judson, Ethan Greenblatt University of British Columbia

Mutations in the fragile X mental retardation 1 (FMR1) gene result in fragile X syndrome (FXS) and fragile X primary ovarian insufficiency (FXPOI) - leading causes of intellectual disability (ID), autism spectrum disorder (ASD) and premature ovarian failure. FMR1 encodes the RNA-binding protein FMRP, which binds to dozens of mRNAs associated with various ASDs. Both neurons and oocytes translationally regulate stored mRNAs that are associated with FMRP. Mature oocytes are large, transcriptionally silent cells which depend entirely on the translation of stored mRNAs, making them a useful model to study FMRP-dependent gene regulation. Our lab previously demonstrated that Drosophila Fmr1 functions in oocytes primarily as a translational activator, and promotes the production of many large proteins essential for neurodevelopment.

We developed a single molecule fluorescent *in situ* hybridization (smFISH)-based assay to identify novel regulators of Fmr1 during Drosophila oogenesis. The mRNA of the Fmr1 target, Poe, is localized to RNP particles in an Fmr1 and translation-dependent manner. The loss of the RNA binding protein Rasputin (Rin), led to the dispersal of these 'Poe-particles,' which we hypothesize is due to reduced Poe translation. We performed co-immunoprecipitation of endogenously tagged Fmr1 from ovarian extracts followed by mass spectrometry. Our results showed an enrichment of Rin in Fmr1-tagged extracts relative to controls. We also identified several other RNA binding proteins as novel Fmr1 interactors, including CG5726, an ortholog of the mammalian non-canonical translation initiation factor CTIF. Previous

studies have led to a proposed role for Rin in the stabilization and translational upregulation of its target mRNAs. We hypothesize that Rin interacts with Fmr1 and CG5726 to facilitate translational activation of Fmr1 targets in oocytes and neurons.

859C Steroid hormone signaling activates a sensory switch during *Drosophila* peripheral nervous system development *Jacob Jaszczak*[1,2], Laura DeVault[1,3], Lily Jan[1,2], Yuh-Nung Jan[1,2] 1) Department of Physiology, Department of Biochemistry and Biophysics, University of California at San Francisco, San Francisco, California, USA; 2) Howard Hughes Medical Institute; 3) Department of Developmental Biology, Washington University Medical School, Saint Louis, USA

Sensory neurons which detect multiple types of stimuli enable animals to sense environmental changes and avoid harm. How the neuronal properties which facilitate sensation are activated during development and the mechanisms which allow neurons to distinguish sensory modalities are poorly understood. The Class 4 dendritic arborization (C4da) neurons are multimodal sensors which tile the body wall of *Drosophila* larvae and detect temperature, light, and mechanical force. The development of the sensory responses mediated by C4da neurons demonstrates that the sensory function and modality are developmentally tuned processes. While larvae respond to mechanical force throughout development (Almeida-Carvalho et al. 2017), a robust activation of the thermal nociceptive escape behavior is only present during the second half of larval development (3rd instar) (Sulkowski et al. 2011). In contrast to the increase in nociceptive behavior in the 3rd instar, we find that ultraviolet light-induced Ca^{2+} activity in C4da neurons decreases during the same period of larval development. This demonstrates that different modalities detected by the same sensory neuron can each have different developmental patterns of activity. The steroid hormone receptor Ecdysone Receptor A (EcR.A) is required for thermal nociception in 3rd instar C4da neurons (McParland et al. 2015); thus, we examined the role of ecdysone in the sensory switch. We find that EcR nuclear localization increases early in the 3rd instar, and demonstrate that both increasing ecdysone titer and overexpression of EcR.A is sufficient to promote precocious thermal nociceptive responses in 2nd instar larvae. Additionally, we find that both 2nd instar and 3rd instar nociception requires EcR.A ligand dependent activation to promote thermal nociception. We next measured the expression of known nociceptive channels. While most of the channels have changing expression from 2nd to 3rd instar, we find EcR.A suppresses expression of *subdued* (encoding a TMEM16 channel) with indiscernible effect on the other nociceptive genes. RNAi reduction of *subdued* expression in 2nd instar C4da neurons not only increases thermal nociception, phenocopying EcR.A overexpression, but also decreases the response to ultraviolet light. Thus, steroid hormone signaling suppression of *subdued* expression facilitates the sensory switch of C4da neurons, and suggests that ion channel balance is a key target for tuning the development of sensory modalities.

860A Genetic regulation and protein interactions necessary for proper formation of *Drosophila* rhabdomeres and the inter-rhabdomeral space *Johnathan Rylee*[1], Simpla Mahato[1], Lalitha Sastry[1], Lauren Feder[1], Shaun Grega[1], Brandon Sizemore[1], Andrew Zelhof[1,2] 1) Department of Biology, Indiana University, Bloomington, IN; 2) Drosophila Genomics Resource Center, Indiana University, Bloomington, IN

Drosophila Eyes Shut (EYS) is responsible for creating and filling the inter-rhabdomeral space (IRS), a unique extracellular matrix that spatially separates the apical rhabdomeres of photoreceptors in each ommatidium, a unique adaptation in higher order Diptera. This organization represents an important step in terminal differentiation of photoreceptors as well as establishing a functional visual system in *Drosophila*. A major goal of our lab is to identify and characterize the genes and protein products necessary for this adaptation. We have taken two approaches towards this goal. First, we carried out an RNAi screen to identify transcription factors necessary for proper rhabdomere and IRS formation. Second, we have identified interactors with EYS through the use of proximity labeling and mass spectrometry. We will present results of both screens and how these newly identified genes contribute to the differentiation and organization of photoreceptors in insects.

861B Molecular instructions for the production of sparse inputs *Vanessa Puñal*[1], Najia Elkahlah[1], Jackson Rogow[2], Jamal Jenkins[1], E. Josie Clowney[1], Mark Lewis[1], Mona Saeed[1], E. Josie Clowney[1] 1) University of Michigan; 2) The Rockefeller University

Detection of environmental stimuli is essential for survival. The total number of sensory stimuli encountered by an organism is unpredictable and nearly limitless, making genetic coding of detectors for all possible stimuli impossible. How then does development build neural circuits capable of processing unforeseen and expansive arrays of sensory input? One strategy is to harness the power of combinations. Consider a palette of 100 colors: when combined, this limited number of colors can paint a rich picture containing ~10^{158} hues. Similarly, evolution has selected for amplifying the number of discriminable stimuli from a limited number of genetically encoded sensors to the number of combinations among them. This is achieved by expanding sensory neuron inputs via sparse, combinatorial wiring to higher order neurons involved in perceptual processing. It is not understood in any species what developmental mechanisms give rise to the input sparseness necessary for sensory amplification. To address this knowledge gap, I use the fly olfactory system where 50 odor channels are dispersed, via Projection Neurons, among ~2,500 higher order neurons called Kenyon Cells in the mushroom body calyx. Previous work in the lab demonstrated that in the absence of Kenyon Cells during

development, adult Projection Neuron axons no longer provide input to the calyx. We have subsequently found that this is because developing Projection Neurons never initiate collateral formation when Kenyon Cells are not present. These results have led to the hypothesis that Kenyon Cells dictate their input density via retrograde feedback to Projection Neurons. To test this hypothesis, we generated a bulk RNA sequencing dataset for developing Kenyon Cells and used this as the basis for a candidate screen to search for Kenyon Cell-derived retrograde signals required for the production of odor channel inputs. Results suggest a role for a variety of molecules secreted by Kenyon Cells as part of a molecular pathway that drives Projection Neurons to meet Kenyon Cell demands to generate odor channel inputs.

862C Analysis of sexually dimorphic gene expression in *Drosophila* legs *Jude Icoy*, Pratyajit Mohapatra, Karen Menuz University of Connecticut, Storrs, CT

Insects have gustatory (taste) sensilla on their wings, labella, and tarsi. These sensilla house gustatory receptor neurons which express gustatory receptors. The *Drosophila* genome contains two known families of gustatory receptors, the Grs and the IRs. Such receptors can be involved in tasting food or conspecific pheromones. Although the expression of these receptors is best-characterized in the labellum, their expression is not as well defined in the legs. There are different combinations of sensilla in the hindlegs, midlegs, and forelegs due to their different roles in behavior. In particular, the forelegs are used by males during courtship. Thus, gustatory receptor expression is expected to be sexually dimorphic and to vary between the different leg-types. Additionally, other genes may also be differentially expressed by leg-type and gender. We have utilized RNASeq to profile gene expression in male and female forelegs, midlegs, and hindlegs. Differentially expressed genes are identified and categorized by gender and by leg-type. This work can inform future studies on the role of sexually dimorphic genes in *Drosophila* gustatory behavior.

863A Elucidating the interaction between the chromatin reader Kismet and histone deacetylases in the promotion of axon pruning *Emily Sterner*[1], Daniel Marenda[2], Jennifer Stanford[1] 1) Drexel University, Philadelphia, PA; 2) Division of Biological Infrastructure, National Science Foundation, Alexandria, VA

In *Drosophila*, the chromatin reader Kismet (Kis) is required for proper axon pruning in the developing mushroom body during pupation. Kis is a homolog of the human transcription factor and chormatin reader CHD7. Haploinsufficiency of CHD7 accounts for two-thirds of CHARGE syndrome cases in humans. This neurodevelopmental disorder causes intellectual disability and defects in facial structure and sensory organs. The axon pruning defect caused by loss of Kis in *Drosophila* can be rescued pharmacologically by HDAC inhibition. Previously, our lab has shown the general HDAC inhibitor SAHA is able to rescue axon pruning defects. To narrow down which HDAC(s) are relevant to the axon pruning process, we are taking a two-pronged approach of: 1) pharmacological inhibition using more specific HDAC inhibitors, and 2) RNAi knockdown of specific HDACs in a *Drosophila* model for CHARGE syndrome. The Class I HDAC inhibitors Beta-hydroxybutyric acid and sodium butyrate are both able to rescue defects caused by loss of Kis, suggesting that Class I HDACs are relevant to axon pruning and could be putative targets for potential therapeutic for CHARGE syndrome. Follow-up work to confirm the involvement of HDAC1 and/or HDAC3 in the axon pruning process, using RNAi, is ongoing.

864B Bisphenol A exposure impacts neurodevelopmental gene expression, cognitive function, and synaptic morphology in *Drosophila melanogaster* *Judith Anderson*[1], Chloe Welch[1], Rana Ghobashy[1], Salma Elliessy[1], Eden Johnson[2], Angelina Tupikova[1], Johnathan Newman[1], Adam Alfareh[1], Erin Widman[1], Alexandra Davis[1], Wendy Lee[2], Kimberly Mulligan[1] 1) California State University, Sacramento; 2) California State University, San Jose

Bisphenol A (BPA) is an environmentally prevalent endocrine disrupting chemical that may be a risk factor for neurodevelopmental disorders. BPA has been associated with behavioral impairment in children and causes a variety of neurodevelopmental phenotypes in model organisms. We used *Drosophila melanogaster* as model to explore the consequences of developmental BPA exposure on gene expression, cognitive function, and synapse development. Following RNA-sequencing, we performed Gene Set Enrichment Analysis (GSEA) and found neurodevelopmentally relevant genes were significantly impacted by BPA (1mM). Among the top misregulated genes were those associated with learning and synapse development. To measure learning, we used a behavioral paradigm called conditioned courtship suppression. In this paradigm, male flies are exposed to an unreceptive female (an “aversive stimulus”) for an hour. Flies with unimpaired learning exhibit a significant decrease in their courtship activity in the final ten minutes compared to the initial ten minutes of the assessment period. We found that BPA-treated flies did not reduce their courtship activity in the final ten-minute interval, indicating that BPA impaired learning. We also used immunofluorescence to examine synapse morphology within the larval neuromuscular junction and found BPA significantly increased the number of axonal branches. Our findings align with studies of BPA in mammalian model organisms, suggesting that BPA impairs functionally conserved neurodevelopmental pathways. Further, because *Drosophila* do not possess classic estrogen receptors, this research indicates that BPA can impact neurodevelopment by molecular mechanisms distinct from its role as an estrogen mimic.

865C Enhancing Mask activity in dopaminergic Neurons extends lifespan in flies Xiaolin Tian LSU Health Science Center

Dopaminergic neurons (DANs) are essential modulators for brain functions involving memory formation, reward processing, and decision-making. Here I demonstrate a novel and important function of the DANs in regulating aging and longevity. Overexpressing the putative scaffolding protein Mask in two small groups of DANs in flies can significantly extend the lifespan in flies and sustain adult locomotor and fecundity at old ages. This Mask-induced beneficial effect requires dopaminergic transmission but cannot be recapitulated by elevating dopamine production alone in the DANs. Independent activation of $G\alpha_s$ in the same two groups of DANs via the drug-inducible DREADD system also extends fly lifespan, further indicating the connection of specific DANs to aging control. The Mask-induced lifespan extension appears to depend on the function of Mask to regulate microtubule (MT) stability. A structure–function analysis demonstrated that the ankyrin repeats domain in the Mask protein is both necessary for regulating MT stability (when expressed in muscles and motor neurons) and sufficient to prolong longevity (when expressed in the two groups of DANs). Furthermore, DAN-specific overexpression of Unc-104 or knockdown of $p150^{Glued}$, two independent interventions previously shown to impact MT dynamics, also extends lifespan in flies. Together, these data demonstrated a novel DANs-dependent mechanism that, upon the tuning of their MT dynamics, modulates systemic aging and longevity in flies.

866V Glia-neuron signaling induced by distinct sources of two different BMPs regulate synaptic growth *Mathieu BARTOLETTI*, Tracy KNIGHT, Aaron HELD, Laura RAND, Kristi WHARTON Brown University

The nervous system is a complex network of cells whose interactions provide circuitry that enables an organism to perceive and move through its environment. Both neuron-neuron and neuron-non-neuronal cell communications are essential for proper nervous system function. Considerable research has focused on communication between motor neurons and muscles, albeit the molecular basis of glial cell-neuron communication is still not well understood and its elucidation will advance our understanding of complex neural responses and abnormalities of the nervous system that arise when such communication fails. In *Drosophilamelanogaster*, retrograde BMP signaling, activated by muscle-expressed Gbb (BMP5/6/7), has been shown to influence synaptic growth, function and plasticity. Here, we report Gbb also acts from glial cells to initiate signaling in motor neurons, an additional source of BMP ligand required for synaptic growth at the neuromuscular junction (NMJ). Furthermore, we find Dpp (BMP2/4), expressed in a subset of motor neurons (RP2 and aCC), facilitates NMJ growth through autocrine neuronal signaling and its expression in RP2 versus aCC motor neurons appears tightly controlled via an autoregulatory loop. Dpp also initiates neuron to glial cell signaling, but not neuron-neuron signaling to neighboring motor neurons. Together, our findings demonstrate that bi-directional communication between glia and neurons requires two different BMP ligands. Gbb, broadly expressed in glia, as well as muscles, and Dpp, from a discrete set of neurons, activate Smad-dependent BMP signaling in motor neurons to control synapse size.

867V Early lineage segregation of the retinal basal glia in the *Drosophila* eye disc *Chia-Kang Tsao*[1,2], Yu Fen Huang[1,2], Y. Henry Sun[1,2] 1) Institute of Molecular Biology, Academia Sinica, Taipei, Taiwan, ROC; 2) Department of Life Sciences and Institute of Genome Sciences, National Yang-Ming University, Taipei, Taiwan, ROC

The retinal basal glia (RBG) is a group of glia that migrates from the optic stalk into the third instar larval eye disc while the photoreceptor cells (PR) are differentiating. The RBGs are grouped into three major classes based on molecular and morphological characteristics: surface glia (SG), wrapping glia (WG) and carpet glia (CG). The SGs migrate and divide. The WGs are postmitotic and wraps PR axons. The CGs have giant nucleus and extensive membrane extension that each covers half of the eye disc. In this study, we used lineage tracing methods to determine the lineage relationships among these glia subtypes and the temporal profile of the lineage decisions for RBG development. We found that the CG lineage segregated from the other RBG very early in the embryonic stage. It has been proposed that the SGs migrate under the CG membrane, which prevented SGs from contacting with the PR axons lying above the CG membrane. Upon passing the front of the CG membrane, which is slightly behind the morphogenetic furrow that marks the front of PR differentiation, the migrating SG contact the nascent PR axon, which in turn release FGF to induce SGs' differentiation into WG. Interestingly, we found that SGs are equally distributed apical and basal to the CG membrane, so that the apical SGs are not prevented from contacting PR axons by CG membrane. Clonal analysis reveals that the apical and basal RBG are derived from distinct lineages determined before they enter the eye disc. Moreover, the basal SG lack the competence to respond to FGFR signaling, preventing its differentiation into WG. Our findings suggest that this novel glia-to-glia differentiation is both dependent on early lineage decision and on a yet unidentified regulatory mechanism, which can provide spatiotemporal coordination of WG differentiation with the progressive differentiation of photoreceptor neurons.

868V Organizing the Drosophila olfactory circuits by interacting Ig superfamily adhesion molecules *Qichen Duan*, Scott Barish, Allison Carson, Rachel Estrella, Pelin Volkan Department of Biology, Duke University, Durham, NC

The Drosophila olfactory system provides an excellent model to study how complex neuronal circuits are assembled. In Drosophila, each olfactory receptor neuron (ORN) class exclusively expresses a unique olfactory receptor (OR) gene and target each class-specific and uniquely positioned glomerulus in the antennal lobe. How ORN axon terminals are organized into these dedicated structural compartments is not well understood. Through transcriptome profiling of

the antennal tissues during development and RNAi screen, we identified two protein subfamilies belonging to the Immunoglobulin Superfamily, Beats and their heterophilic binding partners Sides, as novel regulators of ORN glomerular organization. Many Beats and Sides are expressed at low levels at early pupal stages but increase their expression levels later. Beats and Sides are also differentially expressed across ORN classes, making them good candidates for encoding the ORN class-specific cell surface codes to mediate neuron-neuron recognition. Perturbing the functions of many Beats and Sides in all or a subset of ORNs at later stages leads to diverse local defects of ORN glomerular organization, associated with the expanding, split, or flipped glomerular morphology. Binding Beat-Side pairs are co-expressed in the same class of ORNs and knockdown of either member of these interacting pairs could lead to similar ORN axonal disorganization. These defects are not likely to be resulted from synaptic mismatching, but rather the loss of axonal adhesion. Furthermore, OR function regulates the expression of Beats and Sides, as some of these genes show altered expression levels in OR mutants. Our data suggest the context-dependent control of ORN glomerular organization by Beat/Side combinatorial codes, and bring new insights into how diverse neuronal populations are coordinated into hardwired circuits.

869V Chordotonal neurons have dendritic spike initiation zones that are controlled by Para, the *Drosophila* sodium channel *Thomas A. Ravenscroft*[1,2], Ashleigh Jacobs[3], Mingxue Gu[1,2], Daniel F. Eberl[3], Hugo J. Bellen[1,2,4] 1) Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 2) Jan and Dan Duncan Neurological Research Institute, Texas Children›s Hospital, Houston, TX; 3) Department of Biology, University of Iowa, Iowa City, IA; 4) Department of Neuroscience, Baylor College of Medicine, Houston, TX

The fruit fly *Drosophila melanogaster* has provided important insights into how sensory information is transduced by Transient Receptor Potential (TRP) channels in the Peripheral Nervous System (PNS). In bipolar chordotonal neurons (CN), TRP channels alone are not sufficient for the transduction of mechanical stimuli. We use an intronic *Minos*-mediated integration cassette to show that the sole voltage-gated sodium channel (Na_V) in *Drosophila*, Para, is localized to the dendrites of these bipolar neurons. Para is localized to the distal tip of the dendrites in all CN and is colocalized with the mechanosensitive TRP channel NompC, distal to the mechanosensitive TRP channel Inactive. This localization is unique to bipolar CNs as we do not observe dendritic localization of Para in other peripheral neurons. Reducing para expression using RNAi in chordotonal neurons of the adult Johnston's organ severely affects sound-evoked potentials. However, in addition to the dendritic localization, Para also localizes to a proximal spike initiation zone (SIZ) at an axonal initial segment-like region in axons of both bipolar and multidendritic PNS neurons. Therefore, to determine the dendritic specific role of *para* in CN neurons, we expressed a genetically encoded calcium indicator in CNs and recorded the fluorescence spike changes in dendrites upon mechanical stimuli. We show that the observed spikes in dendrites are nearly completely abolished by exposure to the Na_V inhibitor Tetrodotoxin (TTX). We conclude that Na_V channels are essential for mechanosensation in CN dendrites and that Para localization marks a dendritic SIZ in the distal dendrite as well as a SIZ in axons near the cell body. Hence, the TRP channels present in the dendrite of chordotonal neurons must cooperate with Para to trigger a neuronal response.

870V The post-transcriptional regulation of TFs in immature motoneurons shapes the axon-muscle connectome *WENYUE GUAN*[1], Stéphanie Bellemin[1], Mathilde Bouchet[1], Lalanti Venkatasubramanian[2], Camille Guillermin[1], Anne Laurençon[1], Kabir Chérif[1], Aurélien Darnas[1], Christophe Godin[3], Séverine Urdy[1], Richard S. Mann[2], Jonathan Enriquez[1] 1) Institut de génomique fonctionnelle de Lyon, ENS de Lyon; 2) Mortimer B. Zuckerman Mind Brain Behavior Institute, Columbia University; 3) Laboratoire Reproduction et Développement des Plantes, Univ Lyon, ENS de Lyon

Temporal factors expressed sequentially in neural stem cells, such as RNA binding proteins (RBPs) or transcription factors (TFs), are key elements in the generation of neuronal diversity. The molecular mechanism underlying how the temporal identity of stem cells is decoded into their progeny to generate neuronal diversity is largely unknown. Here, we used genetic manipulations and a new computational tool to study the unique fates of the progeny of a stem cell producing 29 morphologically distinct leg motoneurons (MNs) in *Drosophila*. We identified 19 combinatorial codes of transcription factors (TFs) expressed in immature MNs (iMNs) just before their morphological differentiation. Importantly these TF codes are progressively established as a function of their birth date. The comparison of the RNA and protein expression patterns of 6 TFs in the 29 iMNs in different combinations revealed that post-transcriptional regulation plays an essential role in shaping these TF codes. We found that the two known RBPs, Imp and Syp, temporally expressed in the NB and maintained in iMNs according to their birth date, are key players in the construction of the axon-muscle connectome. Both RBPs post-transcriptionally regulate 5 of the 6 TFs examined, which in turn control the establishment of the axon muscle-connectome. By deciphering the function of Imp in the iMNs with respect to the stem cell, we propose a model whereby these two crucial RBPs act in iMNs to specify the unique morphological fate of individual MNs. Taken together, our study reveals that iMNs have the potential to acquire different morphological fates because of a broad expression of different TF mRNAs. However, these potentials are post-transcriptionally narrowed down to single iMN or subpopulations of iMNs by Imp and Syp, and potentially by other RBPs that remain to be discovered, to ultimately fine-tune the morphological fates of individual MNs.

871V Candidate Autism Genes Nrx1 and Nlg3 Lead To Ectopic Synapses in Nociceptive Neurons in Drosophila Larvae *Claudia Gualtieri*, Fernando Vonhoff University of Maryland Baltimore County

Synaptic refinement is a neuroplasticity process leading to the withdrawal of ectopic synapses formed during the initial phases of neuronal development. Extensive research has shown evidence of synaptic refinement occurring in both the central nervous system (CNS) and peripheral nervous system (PNS). However, the molecular mechanisms underlying synaptic refinement remain incompletely understood. The process of synapse elimination is crucial during development in multiple organisms as it has also been linked to the onset of neurodevelopmental disorders like autism. Evidence from postmortem studies of autistic patients that have mutations in genes associated with autism, show increased synaptic density and longer dendritic spines, suggesting a possible defect in the elimination process. The goal of this study was to determine the anatomical effects of candidate autism genes in vivo using the Drosophila model. Starting from the hypothesis that candidate autism genes would lead to the presence of ectopic synapses that branch off stereotypic connectivity patterns, anatomical synaptic innervations of cIV nociceptive sensory neurons were assessed during larval development. The candidate autism genes nrx-1 or nlg-3, respectively coding for the scaffolding proteins neurexin-1 and neuroligin-3, were downregulated using RNAi constructs. Anatomical defects were assessed by counting the number of ectopic synapses and by measuring the fluorescent area covered by synapses. Preliminary data on the anatomical effects of the downregulation of the candidate autism gene nrx-1 shows increased number of ectopic synapses in the ladder structure formed in the CNS by the axonal projection of nociceptive neurons. These findings offer the basis for investigating the processes leading to the failure in the elimination of ectopic synapses providing insights into the molecular mechanisms regulating synaptic refinement

872V It's not just about physical attraction: Investigating the interaction between HDAC4 and Ankyrin2 in *Drosophila melanogaster* neuronal function *Sarah Wilson*, Silvia Schwartz, Helen Fitzsimons School of Natural Sciences, Massey University, Palmerston North, New Zealand

Histone deacetylase 4 (HDAC4) is implicated in several neurodevelopmental and neurodegenerative diseases that involve deficits in memory and cognition. Increased expression of HDAC4 in the *Drosophila* brain impairs neuronal development and memory, thus *Drosophila* is an ideal model to investigate the molecular pathways through which HDAC4 acts. A recent genetic screen in *Drosophila*, for genes that interact in the same molecular pathway as HDAC4, identified the cytoskeletal adaptor Ankyrin2 (Ank2). Knockdown of Ank2 in the brain resulted in deficits in axon morphogenesis (Fisher's, $p < 0.01$) with reduced elongation and guidance defects as well as significantly reduced dendritic branch lengths (Student's t-test, $p < 0.05$), all of which are similar phenotypes to those resulting from increased expression of HDAC4. HDAC4 contains a putative ankyrin-binding motif, suggesting that it may interact physically with Ank2, however no interaction was detected via co-immunoprecipitation. Further investigation revealed that expression of HDAC4 with a mutated ankyrin-binding motif retained the ability to interact genetically with Ank2 to synergistically impair photoreceptor development (ANOVA, $p < 0.01$), furthermore, this genetic interaction was dependent on the presence of HDAC4 in the nucleus. Together these data show that Ank2 and nuclear HDAC4 indirectly interact to regulate neuronal morphogenesis and function.

873V Identifying New Players in Structural Synaptic Plasticity *Cong Xiao*, Peter M'Angale, Shuhao Wang, Max Zinter, Adrienne Lemieux, Travis Thomson University of Massachusetts Medical School

Synapses are connections between a presynaptic neuron and a postsynaptic cell, proper formation of synapses are needed for neuronal signaling. The ability to make and prune synapses is referred to as structural synaptic plasticity. The perturbation of plasticity is associated with many disease states. We use the Drosophila neuromuscular junction (NMJ) to study synaptic plasticity. Previous work in our lab shows that the Drosophila homolog of Activity-regulated cytoskeleton associated protein (dArc1), a retrovirus-like protein, forms capsids, transfers its own RNA in a viral-like fashion across the NMJ modulates, and finally that this transfer is needed for structural synaptic plasticity. However, the mechanism of how dArc1 regulates plasticity, and how its transfer is involved in this regulation is unknown.
Here, using dArc1 knock downs in motor neurons followed by deep sequencing, we have found a subset of mRNAs that are decreased in muscle .
These represent RNAs that might depend on dArc1 for transfer across the synapse. Using this approach, we found that the Drosophila homolog of *muscleblind* (*mbl*) in fly muscles is decreased significantly with dArc1 knocked down in motor neurons. Consistent with a transfer of *mbl* across the NMJ, over-expression of *mbl* in the neuron causes a significant increase of mbl RNA both pre- and post- synaptically. Further, co-immunoprecipitation experiments suggest dArc1 protein binds to *mbl* RNA. Strikingly, both *mbl* knock down and *mbl*over-expression cause a reduction of structural synaptic plasticity.
Taken together, *mbl* mRNA is transferred across the NMJ and may be a cargo of dArc1 capsids and genetically it appears *mbl* is needed for structural synaptic plasticity. We are now investigating if the known functions of Mbl, such as regulating alternative splicing or RNA localization is the mechanism by which changes in Mbl expression affects plasticity.

874V The Role of Glial Peroxisome in Neuron-Glia Communication in Drosophila *Maggie Sodders*, Hua Bai Iowa State

University

Peroxisome function in glial cells has been shown to impact neuronal functions. In the present study, we aim to elucidate the mechanism behind peroxisome-mediated glia-neuron communication using *Drosophila* as a model organism. I used the GAL4/UAS system to drive RNAi knockdown or overexpression in either glia tissue or motor neurons. The morphology of adult abdominal neuromuscular junction (NMJ) was visualized using confocal microscopy and the axonal volume was quantified via ImageJ. Glial-specific knockdown of peroxisome import protein, Pex5, results in swollen axon as compared to the control. Consistent with our previous work showing that defective peroxisomes upregulate inflammatory cytokine upd3 and JAK-STAT signaling, overexpression of upd3 in glia phenocopies glial-specific knockdown of Pex5 and increases axonal volume and area. We further show that neuronal-specific activation of the JAK-STAT pathway through hop overexpression results in an increase in axon size. Taken together, our findings suggest that impairment of peroxisomes in the glial impacts axonal morphology and potentially functions via inflammatory response, specifically the JAK-STAT pathway.

875V A comprehensive temporal patterning gene network controls developmental timing in Drosophila medulla neuroblasts *Hailun Zhu*[1], Sihai Dave Zhao[2], Alokananda Ray[1], Yu Zhang[1], Xin Li[1] 1) University of Illinois at Urbana-Champaign, Department of Cell and Developmental Biology, Urbana, IL; 2) University of Illinois at Urbana-Champaign, Department of Statistics, Urbana, IL

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. However, these TTFs identified through candidate antibody screening may not compose the complete TTF sequence, and several gaps remained unfilled concerning the initiation, termination and regulation of this temporal cascade. To address these questions, 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. After analyzing the scRNA-seq data of medulla neuroblasts, two sets of genes showed high to low or low to high gradients of expression as neuroblasts age. With known TTFs as markers, we identified a list of novel TTF candidates, among which, SoxNeuro (SoxN), doublesex-Mab related 99B (Dmrt99B), Odd paired (Opa), Earmuff (Erm), Scarecrow (Scro), BarH1, BarH2 and Glial cells missing (Gcm) are necessary for medulla temporal patterning. We identified extensive cross-regulations among these novel TTFs and known TTFs, that generally follow the rule that a previous TTF is required to activate a later TTF, while a later TTF would repress a previous TTF. 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, in pursuit of the mechanism behind the regulation of the temporal cascade, we found that the initiation, progression and termination of the TTF temporal cascade require genes differentially expressed along the differentiation axis (NBs -> -> neurons) including Lola and Nerfin-1, providing clues as to why the temporal progression only happens in neuroblasts but not their differentiated progeny. In summary, our study revealed a comprehensive temporal gene network that controls developmental timing in neural progenitors.

876B Natural genetic modifiers of sensitivity to dopamine-level perturbations in *Drosophila melanogaster* *Ana Marija Jaksic*[1], Andrew G. Clark[2] 1) EPFL Swiss Federal Institute of Technology Lausanne; 2) Cornell University, Ithaca, NY

Dopamine (DA) plays a major role in many animal behaviors, and yet its level is highly variable. It naturally changes over the lifetime in concert with physiological states and environmental stresses. In order to maintain stable expression of many important downstream behaviors, DA level homeostasis needs to be regulated. The way DA homeostasis is achieved on a cellular level has been under extensive investigation, due to its role in Parkinson's disease. However, genetic variation underlying these traits remains relatively unexplored. The extensively characterized genetic diversity existing in the *Drosophila melanogaster* Genetic Reference Panel, as well as the utility of *Drosophila* neurogenetic toolkit, enables us to pursue this question in a systematic way.
Here, we use pharmacological interventions to perturb DA level in diverse genetic backgrounds of the DGRP.This enabled us to simulate exogenously induced dopamine perturbations while avoiding confounding the effects of dopamine level with the systemic phenotypic response to a specific environment. We administered L-DOPA (dopamine precursor) and 3IY (dopamine-precursor agonist) to the DGRP lines and then measured changes in locomotion across genotypes. This enabled us to explore the interaction in 193 genotypes and three dopamine states (nominal, elevated and depleted). Using genome-wide association study we then identified new genetic modifiers of perturbed dopamine phenotypes. These variants point to novel as well as known pathways that affect DA signaling. Namely, we find that the natural genetic variation of enzymes along the cAMP signaling cascade, in the octopamine synthesis, as well as sulfotransferase pathways may play an important role in maintaining locomotion upon perturbation of DA levels. In this study we explore and discuss their functional relevance.

877C Neuronal gluconeogenesis regulates systemic glucose homeostasis via FMRFa signaling *Tetsuya Miyamoto*, Sheida Hedjazi, Hubert Amrein Texas A&M Health Science Center, Bryan, TX

Neuropeptides and their cognate receptors are critical molecular effectors that modulate many physiological processes and behavioral outputs. We recently reported that Glucose-6-phosphatase, one of the key gluconeogenic enzymes, is exclusively expressed in a small subset of about 30 large neurosecretory cells in the CNS of the fruit fly. Moreover, we demonstrated that neural activities of *G6P* neurons, but not glucose production per se, is critical for glucose homeostasis. These neurons are essential to maintain systemic glucose homeostasis, yet their identities are largely unknown. Here we show that *G6P-GAL4* neurons express at least five distinct neuropeptides such as NPF, Orcokinin, PDF, FMRFa and Nplp1. Neural silencing of each class of neuropeptide neurons showed that only the FMRFa-producing neurons in the thoracic ganglion are essential for glucose homeostasis. *FMRFa* mutants show a similar hypoglycemic phenotype as G6P mutants, suggesting that G6P maintains systemic glucose homeostasis via FMRFa signaling. To support this idea, we measured secretory capabilities of FMRFa neurons using a neuropeptide reporter, a mammalian atrial natriuretic peptide fused to GFP (ANF-GFP). We found that *G6P* mutants show significantly lower amounts of ANF-GFP in the hemolymph, compared to *G6P* wildtype flies. These results indicate that G6P cell-autonomously increases circulating neuropeptide amounts by facilitating neuropeptide processing, transport and/or release. Taken together, our data suggest that G6P maintains systemic glucose homeostasis by increasing FMRFa amounts in the hemolymph.

878A Exploring the effects of multiple neuropeptides on state-dependent visuomotor transformations *Avery Krieger*, Ryan York, Luke Brezovec, Thomas Clandinin Stanford University

Effectively acting on the demands of internal states is vital for survival. For example, when an animal experiences hunger, signals from nutrient sensors must be transmitted to its brain to orient its behavior towards acquiring sustenance. The internal state of hunger and the sensory inputs relevant to finding food must be integrated and transformed into action.

Neuropeptides signal on a variety of scales and change the dynamics of neural circuits (Bargmann 2012). The majority of neuropeptide functions are unknown, yet their purported mechanisms make them well suited to signal internal state.

Recent advances allow for specific genetic access to around 40 neuropeptide producer and receptor neuron populations (Deng 2019). By placing these flies in a 1x1 meter enclosed environment and tracking their movement, we can precisely track individuals during behavioral bouts. An automated fly dispenser allows for the testing of various genotypes and conditions. Screens completely surrounding the fly allow for both static and dynamic visual stimulus presentation. To test the effects of various neuropeptide neurons on behavior, we activate them via optogenetic stimulation during the trial. This system allows for the first unbiased behavioral screen of the effects of a large variety of neuropeptides in response to stimuli, as well as a comparison between those effects and that of behavioral and physiological states of individual flies.

Through measurements of the fly's position and heading, we quantify various behavioral metrics of individuals in response to visual stimuli, states, and neuropeptide neuron stimulation. We have found that internal states such as hunger and mated status of flies lead to dramatic changes in the animal's locomotor behavior and that state changes alter the relationship between behavior and visual stimuli. Optogenetically activating the neuropeptide neuron populations also alters locomotor behavior generally and its relationship to visual stimuli, sometimes with similar effects to changing internal states.

These experiments enable us to probe the effects of many neuropeptides and states on behavior. The goal is to characterize the effect of neuropeptides in interpretable ways as they reflect and manipulate internal states of the animal. The ultimate goal is to build a circuit-level understanding of neuropeptide and state computations that transform sensory inputs into behavior, helping animals reach their goals.

879B Molecular mechanism glia use to contribute to the production of motor outputs in *Drosophila* *Rebecca McAvoy*, W. Daniel Tracey, Stephanie Mauthner Gill Center for Biomolecular Sciences and Department of Biology, Indiana University, Bloomington, IN

Glial cells are essential components of the nervous system that shape neural circuits underlying behavior. Many previous studies have found an important function for glia in the regulation of extracellular ion concentrations that affect neuronal excitability. To better understand how glial effects on ion gradients might affect neurons, we manipulated glial ion gradients using the GAL4/UAS system. Specifically, we expressed the light-gated anion channel GtACR1 or the thermal-gated cation channel dTRPA1 in glia and recorded immediate motor behaviors that occurred following light or thermal activation of the channel. Interestingly, a full body contraction was seen upon activating GtACR1 in glia. In contrast, activating dTRPA1 in glia triggered an apparently opposite behavior of full body relaxation and complete immobility. To rule out the possibility that leaky ion channel expression in neurons was the basis of these motor behaviors, we used GAL80 to prevent neuronal populations from expressing GtACR1 or dTRPA1. In both cases, the behaviors persisted,

supporting that glia (and not unintended neuronal expression) contribute to the motor behaviors. To identify which glial subtype(s) produced the accordion phenotype and full body relaxation, we expressed GtACR1 and dTRPA1 in various glial populations. We found that GtACR1 expression in the perineurial and subperineurial glia produced the accordion phenotype, and dTRPA1 expression in the cortex, ensheathing, and wrapping glia produced the full body relaxation. Our results support that changes in glial ion gradients allow glia to directly influence the production of motor outputs.

880C A non-nuclear NF-κB modulates behavioral alcohol sensitivity but not immunity Thilini Wijesekera, Zheng Wu, Nicole Stephens, Rahul Godula, Linda Kao Lew, *Nigel Atkinson* The University of Texas at Austin

NF-κB proteins are well known as transcription factors important in immune system activation. In this highly conserved role, they contribute to changes in behavior in response to infection and in response to a variety of other insults and experiences. In some mammalian neurons, NF-κBs can be found at the synapse and translocate to the nucleus when activated by synaptic activity. In these areas, NF-κBs are believed to produce change by acting in the nucleus to alter gene expression. Here we demonstrate that in *Drosophila melanogaster*, NF-κB action is important both inside and outside the nucleus and that the *Dif* gene has segregated nuclear and non-nuclear NF-κB action into different protein isoforms. The DifA isoform is a canonical nuclear-acting NF-κB protein that enters the nucleus and is centrally important for combating infection. The DifB variant, but not the DifA variant, is expressed in central nervous system neurons. DifB does not enter the nucleus and co-localizes with a synaptic protein. Mutations specific to DifB alter alcohol behavioral sensitivity but have no obvious effect on combating infection, whereas DifA mutants do not affect alcohol sensitivity but compromise the capacity to combat infection. These data are evidence that the unusual, non-nuclear DifB variant, alters behavior by a synaptic mechanism that is local and nongenomic and that diverges from the canonical NF-κB transcriptional effects used in the peripheral immune system. The Drosophila DifB variant is an ideal system for studying non-nuclear NF-κB effects in isolation from NF-κB action in the nucleus.

881A The functionally conserved neuronal pseudokinase Allnighter retrogradely regulates homeostatic UPR and autophagy responses in photoreceptor neurons. *Shashank Shekhar*, Andrew T Moehlman, Minh-Nguyet Hoang, Charles Tracy, Helmut Krämer UT Southwestern Medical Center

Abstract:
Homeostatic responses are essential for neuronal circuits to adapt to changes in the environment. To investigate the molecular mechanisms controlling homeostatic responses in neurons we use a reversible in-vivo model [1]. Extended exposure to ambient light triggers homeostatic responses including reduced rhabdomere size and loss of synaptic active zones in *Drosophila* photoreceptor neurons. These responses are reversible and therefore offer an excellent opportunity to study signaling events controlling neuronal homeostatic responses. Here, we identify *allnighter* (*aln*) as a novel gene required for the regulation of these responses. *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 lose photoreceptor structural integrity and postsynaptic responses after prolonged exposure to ambient light. This response is transient, and photoreceptors recover when returned to LD. Phenotypes are rescued by expression of Aln or its human homolog, the divergent protein kinase FAM69C, independent of its kinase activity. Aln is expressed in many neurons but, surprisingly, not in photoreceptors. Instead, it is secreted by lamina neurons, most prominently L4, and retrogradely regulates photoreceptor responses including UPR and autophagy, two key pathways of homeostatic responses. Perturbation of photoreceptor output by expression of Shi^{ts1} or Tetanus Toxin light chain drastically decreased Aln expression in L4 lamina neurons, and other brain regions. This suggests that the Aln is part of feedback loop in the Drosophila visual system. Aln homologs are highly expressed in mammalian brains suggesting important yet to be discovered functions of these molecules in the mammalian brain.
1. Moehlman, A.T., Casey, A.K., Servage, K., Orth, K., and Krämer, H. (2018). Adaptation to constant light requires Fic-mediated AMPylation of BiP to protect against reversible photoreceptor degeneration. eLife *7*, pii: e38752.

882B The CHD protein, Kismet, regulates both clathrin-mediated and activity-dependent bulk endocytosis at the *Drosophila* neuromuscular junction Emily Hendricks, Taylor Delaney, *Faith Liebl* Southern Illinois University Edwardsville

Chromodomain helicase binding domain (CHD) proteins, including CHD7 and CHD8, remodel chromatin to alter transcriptional programs. Both proteins are important for proper neural development as heterozygous mutations in *Chd7* and *Chd8* are causative for CHARGE syndrome and correlated with autism spectrum disorders, respectively. Their roles in mature neurons are poorly understood despite influencing the expression of genes required for cell adhesion, neurotransmission, and synaptic plasticity. The *Drosophila* homolog of CHD7 and CHD8, Kismet (Kis), promotes neurotransmission, endocytosis, and larval movement, and restricts synaptic levels of cell adhesion molecules. Endocytosis is essential in neurons for replenishing synaptic vesicles, maintaining protein localization, and preserving the size and composition of the presynaptic membrane. Endocytosis can occur via clathrin-mediated endocytosis (CME), which is coupled with neural activity and is the most prevalent form of synaptic endocytosis, and activity-dependent bulk endocytosis, which occurs during periods of intense stimulation. We assessed transcripts important for endocytosis

in *kis* mutant central nervous systems. *AP2α*, *dap160/intersectin*, and *endophilin B* (*endoB*), required for CME, show differential expression in *kis* mutants. *Rab11*, *akt, PI3K92E*, and *sgg/GSK3β*, required for ADBE, exhibit reduced transcripts levels. Finally, *syndapin* transcripts, required for both CME and ADBE, were reduced while several other transcripts were unaffected by mutations in *kis*. We were able to restore *endoB*, *PI3K92E*, *rab11*, and *sgg* transcripts in *kis* mutants by expressing *kis* in either neurons or postsynaptic muscle. These data indicate that Kis contributes to both CME and ADBE. We further tested this hypothesis by pharmacologically inhibiting either CME or ADBE in *kis* mutants and examined endocytosis both electrophysiologically and functionally. Collectively, our data indicate that Kis promotes both CME and ADBE by promoting transcription of several endocytic genes.

883C Investigating the Effects of Rab11 on Synaptic Proteins FasII and APPL in *kismet* Mutants *Ireland Smith*, Joshua Preston, Faith Liebl Southern Illinois University Edwardsville

Chromodomain Helicase DNA-Binding Domain (CHD) proteins are chromatin remodelers that are critical for early neuronal development and the expression of genes important for synaptic plasticity. Mutations in CHD7/8 can lead to a variety of disorders in humans such as CHARGE syndrome, a neurodevelopmental disorder, and autism spectrum disorders. Mutations in *kismet (kis)*, the *Drosophila* ortholog of CHD7/8, lead to an accumulation of cell adhesion molecules and the transmembrane protein amyloid precursor protein-like (APPL) at the synapse as well as reductions in endocytosis, synaptic transmission, and larval locomotion. Kismet regulates *rab11* expression. Rab11 is a GTPase that traffics vesicles from the recycling endosome to the plasma membrane. Because of its role in membrane trafficking, Rab11 might facilitate some of these phenotypic changes observed in *kismet* mutants.

To investigate this possibility, we expressed constitutively active or dominant negative isoforms of Rab11 and examined Fasciclin II (FasII), a cell adhesion molecule. Similar to *kis* mutants, constitutively active Rab11 increased synaptic FasII. Expression of dominant negative Rab11, however, decreased FasII protein levels. When *rab11* was knocked down, we found a decrease in APPL, which directly contrasts *kis* mutants that show an increase in APPL levels. In addition, when Rab11 dominant negative and constitutive active isoforms were expressed, we saw no change in endocytosis or endocytic proteins Endophilin A (EndoA) and Dynamin (Dyn) unlike *kis* mutants, which show reductions in each. Kis affects genes, possibly *rab11*, that localize synaptic proteins to phosphatidylinositol-4,5- P_2 (PIP_2), a phospholipid that helps organize proteins into lipid rafts in the plasma membrane. When *rab11* was knocked down, we did find a reduction in PIP_2 levels at the synapse. These data may suggest that while Rab11 might only play a minor role in contributing to *kis* mutant phenotypes, Rab11 may be able to restore *kis* mutant phenotypes. Therefore, we will express wildtype Rab11 in *kis* mutants to determine whether Rab11 can restore the synaptic levels of FasII and APPL.

884A The *Drosophilla* CD63-related tetraspanins, Tsp42Ee and Tsp42Eg, regulate synaptic structure, function, and vesicle pool dynamics *Emily Hendricks*, Faith Liebl Southern Illinois University Edwardsville

Tetraspanins are a well-conserved class of transmembrane proteins that play a regulatory role at the cell membrane by mediating the spatiotemporal distribution of their binding partners—often receptors, cell adhesion molecules, and intracellular signaling proteins. The tetraspanin, CD63, is a classical marker of exosomes and late endosomes and likely regulates vesicular traffic through the exocytic pathway. However, CD63's role in mediating endocytic and exocytic dynamics at the synapse is not well described. Thus, we aim to use the *Drosophila* neuromuscular junction (NMJ) to characterize the role of CD63 orthologs, Tsp42Ee and Tsp42Eg, in regulating synaptic vesicle dynamics. We find that CD63-related tetraspanins negatively regulate endocytosis, likely through control of synaptic vesicle pool dynamics and localization of the synaptic proteins, Endophilin A and Dynamin. We also find that Tsp42Ee and Tsp42Eg regulate synaptic morphology and function as both *tsp* mutants show reductions in locomotor function and mEJC frequency. Furthermore, *tsp42Eg* loss of function mutants show reduction in quantal content and eEJC amplitude suggesting an overall reduction in synaptic vesicle release. These alterations in synaptic function may, at least in part, be attributed to disruptions in cytoskeletal structure as we observe an increase in Futsch loops at *tsp* mutant synapses. Together, these findings implicate CD63-related tetraspanins as overarching regulators of NMJ structure and synaptic vesicle pool dynamics. We will further explore this work by attempting to rescue the *tsp* mutant phenotype through expression of human CD63 in the *tsp* mutant backgrounds. These experiments will aim to validate the functional redundancy of hCD63 in *Drosophila* and further clarify the role that tetraspanins play at the synapse.

885B Uncovering the Genetic Basis of Variation in Learning and Memory Phenotypes using the Drosophila Synthetic Population Resource *Victoria Hamlin*, Huda Ansaf , Patricka Williams-Simon , Elizabeth King University of Missouri

Learning and memory are complex traits in which many genes and regulatory regions affect the phenotypic output of an individual. For animals, these traits are vital functions necessary for adapting to and surviving in an ever-changing environment. Within a given population, there is variation between individuals in their ability to perform these functions, however, the mechanisms underlying this variability are still largely unknown. In addition, some genotypes may show more variable performance than others. Utilizing variance quantitative trait loci (vQTL) mapping, we can identify regions of genetic variation associated with differences in the variability of learning and memory performance in the Drosophila

Synthetic Population Resource (DSPR). This multiparent population consists of about 1800 recombinant inbred lines (RILs) which provides us with ability to perform high resolution genome wide scans to identify quantitative trait loci with significant contribution to the residual variability in learning and memory performance. To test the performance of RILs, flies underwent operant conditioning for place and olfactory learning and memory. In the aversive place learning assay, flies were placed in a heat box that would increase to an intolerable temperature when flies crossed into one side of the chamber followed by testing to see if they remained on the cool associated side after training. In the appetitive olfactory assay, flies are starved for eighteen hours then provided a sugar reward paired with an odor in training followed by a Y-maze choice test to see if they selected the positive conditioned odor. Using a double generalized linear model to detect both mean and variance QTLs, we identified several QTL influencing these key phenotypes.

886C Investigating the role of tRNA methyltransferase ALKBH8 in learning and memory *Shanzeh Sayied*[1], Kim Madhwani[1], Caley Hogan[2], Kate O'Connor-Giles[1, 3] 1) Brown University; 2) University of Wisconsin-Madison; 3) Carney Institute for Brain Science

Regulation of neuronal gene expression is crucial for proper neuronal development and function and occurs at numerous steps, from DNA methylation to protein degradation. Transfer RNAs (tRNAs) are emerging as an important point of regulation. tRNAs are heavily post-transcriptionally modified to regulate tRNA structure and stability and strengthen anticodon-codon interactions. Recently, variants in tRNA-modifying enzymes have been associated with multiple neurological disorders. In particular, mutations in ALKBH8, which encodes a highly conserved tRNA methyltransferase, have recently been linked to intellectual disability in four families. While ALKBH8 has been studied in the context of oxidative stress, its role in the nervous system remains unknown. Due to its role in translation and link to intellectual disability, we hypothesized that ALKBH8 may promote the translation-dependent processes of learning and memory. We generated ALKBH8 null mutants and analyzed sensory memory using a taste aversion assay. The taste aversion assay measures a reduction in the sucrose-induced proboscis extension reflex (PER) after starved female flies learn to associate sucrose with the aversive tastant quinine. Our findings reveal a deficit in the formation of aversive taste memories in ALKBH8 null mutants. These results indicate learning deficits consistent with intellectual disability in humans and establish Drosophila as a model for understanding the underlying mechanisms of ALKBH8-associated intellectual disability.

887A Utilizing Y-mazes to Investigate Olfactory Learning Phenotypic Variations in Drosophila *Huda Ansaf*, Victoria Hamlin, Elizabeth King University of Missouri, Columbia, MO

Fruit flies, and other animals, can navigate in a complex chemosensory environment. Certain odors can act as signals for food, danger, or as pheromones released by conspecifics to elicit innate behavioral responses. Since not all odor stimuli are informative, the brain must make sense of the complexity of the odor signals and interrupt their actual relevance. Fruit flies perceive odors through olfactory sensory neurons that are located in the sensilla on the third antennal segments and the maxillary palps, which exhibit diverse morphological types. The olfactory system of Drosophila resembles systems found in vertebrates in its overall anatomical organization but is significantly reduced in terms of cell number, which makes it an ideal model system to investigate odor processing in the brain. Associative learning represents one process by which new or altered relevance is assigned to a stimulus through experience. For example, an odor that is repetitively paired with a food reward becomes attractive while an odor that often occurs simultaneously with a punishment becomes a predictor for a negative situation and will be avoided in the future. *Drosophila melanogaster* can easily perform such learning and memory tasks and represents an excellent organism to investigate the neuronal mechanisms that control olfactory learning processes. In this work, we identify phenotypic variations based on learning and memory abilities among different recombinant inbred lines (RILS) in a mapping population. Operant conditioning is induced by presenting an odor with or without the availability of a sucrose reward to condition odors as positive (sugar associated) or negative stimulus. During the testing phase, the Drosophila are simultaneously presented with the previously conditioned CS+ and CS- odors. After sufficient time has passed, fly distribution is recorded to allow for associative appetitive conditioning to be reliably measured without bias due to innate preferences. Various control experiments are also done to test whether all genotypes respond normally to odor and light alone. Learning and memory tasks are tested using a custom-built Y-maze apparatus. After repeating Y-maze testing three times, we identified high and low learning phenotype groups and high and low memory phenotype groups.

888B A survey of *cis*-regulatory fragments from the *dissatisfaction* gene identifies a subpopulation of abdominal interneurons that regulate the opening of the vaginal plates during courtship *Julia Diamandi*[1], Julia Duckhorn[1], Jessica Cande[2], Troy Shirangi[1] 1) Villanova University, Villanova, PA; 2) Cooley LLP, Boston, MA

In *Drosophila*, virgin females carrying mutations in the *dissatisfaction* gene (*dsf*) are delayed in mating with males and exhibit deficits in opening their vaginal plates during courtship. *Dsf* contributes to female behavior in part by functioning in a sexually dimorphic population of interneurons in the abdominal ganglion called the ddag neurons. The ddag neurons in females are composed of a variety of neuroanatomical subtypes, but which subtypes matter for female behavior is unknown. Here, we screened seven *cis*-regulatory fragments from the *dsf* gene for reporter expression in *dsf*-

expressing neurons in the larval and adult central nervous system. We find that most of *dsf's* expression in the central nervous system is driven by *cis*-regulatory sequences within *dsf's* third intron. Using these fragments, we identified a subpopulation of local female-specific ddag interneurons that is sufficient for the opening of the vaginal plates in virgin females during courtship. The regulatory fragments targeting this subpopulation of neurons contain putative binding sites to proteins encoded by the sex determination gene, *doublesex*, suggesting that *doublesex* may directly regulate *dsf* expression in a subset of ddag neurons. Our results provide new insights into the neural circuits that mediate the opening of the vaginal plates and the mechanisms that regulate *dsf* expression in the central nervous system.

889C Pleiotropy and the rapid coevolution in reproductive traits in *Drosophila* *Mehrnaz Afkhami*, John Masly
University of Oklahoma

Reproductive traits are some of the most rapidly evolving traits within species, and some of the most rapidly diverging traits between nascent species. Sex-specific enhancer pleiotropy can provide a base for male and female coevolution. Here, we study two genes, *Pox neuro* (*Poxn*) and *CG14567*, that specify the morphology of a male-specific reproductive structure. The epandrial posterior lobes (ePLs) are novel reproductive structures on the male genitalia that are structurally important for mating success. In addition, morphological variation in shape and size of the ePL affects the male's mating success and mating position. When males with a smaller or misshapen posterior lobe mate with a normal female, the female reduces her egg-laying amounts. Previous studies show that *Poxn* directs aspects of male courtship behavior and genital development, and recent studies from our lab show that variation in expression level of *CG14567* in the developing ePL primordia directs variation in genital morphology among members of the *D. melanogaster* species complex.
Interestingly, one *Poxn* enhancer region that directs expression in the developing male genitalia is also expressed in three neurons in the adult female abdominal ganglion. These three neurons enervate the female reproductive tract as well as regions of the gastric tract. This *Poxn* enhancer is also expressed in collections of cells throughout the female VNC and Brian, but compared to males the domain of expression is much reduced. Our preliminary *in-situ* experiments show that *CG14567* is also expressed in the brain and VNC of adult females and appears to localize to these *Poxn*-expressing neurons. We are currently working on behavioral assays to pinpoint the exact function of three neurons in the abdominal ganglion and we are using the GFP Reconstitution Across Synaptic Partners (GRASP) technique to identify the presynaptic cell(s) to these neurons in the females. Our initial functional genetic experiments show that both *Poxn* and *CG14567* expression in these neurons affect female reproduction. In particular, overexpression of *CG14567* in *Poxn* expressing cells increases the eggs laid by the female. Our preliminary genetic and behavioral data suggests that enhancer pleiotropy at *Poxn* and *CG14567* could contribute to coevolution of reproductive traits.

890A Effects of L-DOPA on D. simulans and D. sechellia Mating Behavior *Alyssa Cortés*, Sofia Pogliano, Joseph Coolon, Ivy Lam, Sadie Gregory, Charlotte Freeland, Charity Russell Wesleyan University

The absence of mating between groups of organisms is one criterion for the separation of species. There could be multiple reasons for this absence, including geographical, behavioral, and genetic causes. The fruit flies Drosophila sechellia and Drosophila simulans inhabit the same geographical space, the Seychelles archipelago, yet remain separate species due to considerably low levels of hybridization. Previous studies have shown that their lack of interspecies mating is due to male mate discrimination, and that this mate choice is made by discrimination against cuticular hydrocarbon (CHC) profiles, which are unique to the two species. Additionally, it has been shown that chemicals uniquely present in D. sechellia's food source, Morinda citrifolia (eg L-DOPA), affects the expression levels of genes related to CHC production. Given the likely effects of these species' differing diets, and more specifically their consumption of L-DOPA on their CHC profiles, and the importance of these profiles in male mate choice, we investigated the consequence of dietary L-DOPA on intraspecific and interspecific mating behaviors. Here we show that L-DOPA does affect mating behavior, and this is mostly seen in D. sechellia flies, those that regularly consume L-DOPA in the wild. These results reveal that mating seen among these flies in the wild is influenced by the presence of L-DOPA. Given this, L-DOPA should be included in laboratory studies regarding mating behavior to mimic the flies' natural conditions. Including L-DOPA would create a more natural environment and lead to mating behaviors more like those produced in the wild, generating more accurate results. Our results could be indicative of complex interactions between the environment and the genetic basis of mating behaviors, and thus support motivations for more in-depth studies of the effect of L-DOPA on various genes and the overall CHC profiles of D. simulans and D. sechellia. This would further clarify the mechanisms of their speciation.

891B Impact of histamine deficiency on accessory gland secondary cell differentiation, persistence, and post-mating responses in *Drosophila melanogaster* *Cazmir Sarnacki*[1], Justin Lilley[1], Ryan Blackmer[1], Jonathan Wassink[1], Erika Spafford[1], Jordan Yokubonus[1], Eric Gonzales[1,2], Lauren Gerritson[1], Anna Prince[1], Martin Burg[1,2] 1) Dept. of Biomedical Sciences, Grand Valley State University, Allendale, MI; 2) Dept. of Cell & Molecular Biology, Grand Valley State University, Allendale, MI

The study of histamine and its function in *Drosophila melanogaster* has primarily focused on its role as a neurotransmitter used by photoreceptors[1] and its effects on a number of other behaviors, such as grooming, thermal

preference, and sleep[2]. Recently, we have identified histamine-like immunoreactivity in a vacuole like compartment (VLC) of secondary cells in the male accessory gland. This immunoreactivity is eliminated by null mutations in the *Hdc* gene that encodes the enzyme histidine decarboxylase[3]. Previous studies have shown that secondary cells are responsible for enabling a number of post-mating responses including increased egg laying, enhanced sperm viability, and reduced female receptivity to copulation[4]. For this analysis, Oregon-R female flies were conditioned with either wild-type, Hdc^{JK910}, or Hdc^{P211} males, followed by wild-type males at 1, 4, and 10 days after the conditioning copulation. In the current study, we demonstrate that elimination of histamine via the Hdc^{P211} or Hdc^{JK910} mutations appears to alter the female receptivity to copulation 1 day after copulation, inceasing receptivity when compaared to wild-type. To determine whether the loss of histamine was affecting this phenotype by disrupting the differentiation of secondary cells (or altering their numbers), we examined wild-type, Hdc^{JK910}, or Hdc^{P211} male accessory glands in which secondary cells were marked by GFP labeling. The wild-type and mutant flies were examined at several different ages for the presence of histamine, *dlg* protein as well as GFP to determine whether large deviations were observed in the number of secondary cells or in the patterning of the accessory gland. Results for both mutant *Hdc* alleles indicate that secondary cells are still able to differentiate when histamine is absent in the accessory gland, but the number of cells present appears to be consistently reduced when compared to wild-type flies.

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AP was supported by a GVSU S3 William Schroeder Undergraduate Endowed Fellowship in Chemistry and JW by a GVSU S3 Fellowship for GVSU.

892C Mechanisms of *D2R* signaling in the blood-brain barrier that regulates courtship in *Drosophila melanogaster* *Sumit Gautam*, Cameron R. Love, Brigitte Dauwalder University of Houston, Houston, TX

The blood-brain barrier (BBB) is a highly selective cell layer that separates the nervous system and the circulating hemolymph. The BBB protects the brain from the contents of the circulating fluids that could impede neuronal function. Two layers of glial cells, Perineural Glia (PG) and Subperineural Glia (SPG) make up the BBB in *Drosophila melanogaster* which ensheathes the brain. We have previously shown that adult feminization of BBB cells in male *Drosophila* leads to reduced courtship levels.[1] In a microarray screen of isolated BBB cells we have identified several male-enriched transcripts.[2] One of them encodes the *Dopamine-2 like receptor (D2R).* We discovered 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 are interested in understanding the downstream signaling of D2R in the BBB of *Drosophila* that mediates the effect on courtship. We are examining biased signaling by the D2R receptor by mutagenizing D2R receptor residues that favor G protein or arrestin signaling, respectively. These constructs will be tested for their ability to rescue the D2R courtship defects when they are expressed in adult male *Drosophila* BBB. This will help us better understand how D2R contributes to the regulation of courtship in *Drosophila*.

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893A Regulation of sexually dimorphic abdominal courtship behaviors in *Drosophila* by the *Tlx/tailless*-like nuclear receptor, *Dissatisfaction* *Julia Duckhorn*[1], Jessica Cande[2], Mary Metkus[1], Hyeop Song[1], Sofia Altamirano[1], David Stern[2], Troy Shirangi[1] 1) Villanova University, Villanova PA; 2) Janelia Research Campus, Ashburn VA

Sexually dimorphic courtship behaviors in *Drosophila melanogaster* develop from the activity of the sexual differentiation genes, *doublesex* (*dsx*) and *fruitless* (*fru*), functioning with other regulatory factors that have received little attention. The *dissatisfaction* gene (*dsf*) encodes an orphan nuclear receptor homologous to vertebrate *Tlx* and *Drosophila tailless* that is critical for the development of several aspects of female- and male-specific sexual behaviors. Here, we report the pattern of *dsf* expression in the central nervous system and show that the activity of sexually dimorphic abdominal interneurons that co-express *dsf* and *dsx* is necessary and sufficient for vaginal plate opening in virgin females and abdominal curling in males during courtship. We find that *dsf* activity results in different

neuroanatomical outcomes in females and males, promoting and suppressing, respectively, female development and function of these neurons depending upon the sexual state of *dsx* expression. We posit that *dsf* and *dsx* interact to specify sex differences in the neural circuitry for dimorphic abdominal behaviors.

894B A *Drosophila* model for understanding the perception and central processing of chronic social isolation Wanhe Li Texas A&M University

Social isolation and loneliness have potent effects on public health. Social psychologists have suggested that compromised sleep quality is a key factor that links persistent loneliness to adverse health conditions. We report that chronic, but not acute, social isolation reduces sleep in *Drosophila*. We use quantitative behavioral analysis and transcriptome profiling to differentiate brain states under acute vs. chronic social isolation. Despite animals' uninterrupted access to food, chronic social isolation altered the expression of metabolic genes and induced a brain state that signals starvation. Chronically isolated animals exhibit sleep loss accompanied by overconsumption of food, which resonates with anecdotal findings of loneliness-associated hyperphagia in humans. Chronic social isolation reduces sleep and promotes feeding through neural activities in the fan-shaped body columnar P2 neurons of the fly. Activation of these neurons causes misperception of acute social isolation as chronic social isolation and thus results in sleep loss. These and other new results reveal mechanistic links between chronic social isolation, metabolism, and sleep, presenting a new genetic model to understand the perception and central processing of chronic social isolation.

895C Toll Family Receptor Function in Neuronal Recognition of Immune State *Tim Lebestky*, Nicole Alvarez, Minjun Kim, Raphael Rakosi-Schmidt Williams College

The Toll family of receptors possess a number of critical functions in Drosophila, including roles in development and immunity. Recently, our group has begun looking for behavioral roles for the Toll family of receptors in the nervous system, given a breadth of expression in the adult nervous system. We hypothesize that Toll receptors function as an immune sensors in neurons to communicate immune challenge and infection and triggers behavioral responses for sickness behaviors in Drosophila. We will present our findings primarily on Toll1 and Toll7 with regards to sleep and feeding behaviors and their potential relationship to immune state when challenged by Metarhizium anisopliae, entomopathic fungi known to trigger robust responses from the Toll1 pathway. We will also share behavioral characterization of sleep and feeding behaviors for wild type animals exposed to M. anisopliae. We believe that immune sensors in the nervous system are essential for coordinating immune-behavioral responses in both sensing presence of pathogens as well as circuit-specific responses during chronic illness.

896A The *Drosophila* serotonin transporter (dSERT) is required for proper sleep amount and sleep architecture *Elizabeth Knapp*[1], Henrike Scholz[2], Jeffrey Donlea[3], David Krantz[1] 1) University of California, Los Angeles, Psychiatry, Los Angeles, CA; 2) University of Cologne, Animal Physiology, Köln, Germany; 3) University of California, Los Angeles, Neurobiology, Los Angeles, CA

Sleep is a critical process essential for life and is evolutionarily conserved from insects to mammals. Although sleep disruption has been linked to a variety of neurological and psychiatric disorders, the cellular mechanisms and neural circuitry involved in sleep regulation are not well understood. The biogenic amine serotonin (5-hydroxytryptamine, 5-HT) functions as a key neuromodulator of sleep behavior in both *Drosophila* and mammals. The relationship between serotonergic signaling and sleep has been studied for several decades, however its complex role in sleep regulation remains uncertain, with many studies showing it to play a role in both wakefulness and conversely sleep propensity. These complexities are further compounded by the fact that many antidepressants and antianxiety medications that work through selective inhibition of serotonin reuptake (SSRIs) have been shown to produce contradicting and various effects on sleep, ranging from insomnia to daytime somnolence.
In both *Drosophila* and mammals reuptake of serotonin from the synaptic cleft is mediated via the serotonin transporter (SERT). The relationships between sleep, neurological disorders, and SSRI medications strongly suggest that variations in extracellular serotonin levels as a result of increased or decreased SERT activity could play a key role in modulating sleep behaviors. The molecular mechanisms underlying this process, however, are not well understood in either humans or model systems.
In this study, we use *Drosophila* as a model system to study the mechanisms by which altering *Drosophila* SERT (*dSERT*) activity impacts sleep behavior. Here we use novel *dSERT* mutants to demonstrate that *dSERT* is required for regulating both proper sleep amount and architecture. In addition, we discovered the increased sleep drive exhibited in these *dSERT* mutants is differentially impacted by certain circadian and environmental factors. Lastly, our data suggests that distinct serotonergic circuits modulate daytime and nighttime sleep behaviors independently. Overall, our work provides new insight into the molecular mechanisms of neuromodulation in the context of sleep and enhances our understanding of how serotonergic signaling is involved in sleep behavior.

897B *rhodopsin 3* regulates circadian periodicity *Menglin Li*, Geoff Meyerhof, Kara Simones, Anvitha Aluri, Craig Montell University of California, Santa Barbara

Circadian rhythms are 24 hour cycles that align behavior, physiology, and gene expression with predictable daily changes in the environment. The primary entrainment cue for endogenous circadian clocks is light. Herein, we sought to test the impact of individual rhodopsins, the light-sensing G-protein-coupled receptors primarily expressed in the eye, on circadian rhythms. We found that flies harboring mutations in *rhodopsin 3* (*rh3),* which is expressed in a subset of R7 photoreceptor cells, have an elongated circadian periodicity. Under constant darkness, wild-type flies maintain a locomotor rhythm with a 24 hour periodicity, whereas the rhythm of *rh3* flies is dramatically extended to ~28 hours. This defect in *rh3* flies suppresses the morning and evening anticipatory locomotion that usually precedes changes from lights on to lights off. Furthermore, the timing of sleep and feeding is also disrupted in *rh3* flies. These defects in circadian behavior are associated with a decreased amplitude of mRNA expression of core-clock components in the periphery, as well as a delay in the phase of *clock* mRNA expression. When we housed *rh3* flies under a 28 hour light:dark cycle (14h light:14h dark), the timing of their sleep and feeding was restored, and their lifespan was extended. Our future work aims to map the neuronal connections linking *rh3*-expressing photoreceptor cells and the core clock neurons in the brain, which set 24 hour locomotor rhythms. In conclusion, we have shown that *rh3* is required for 24 hour circadian periodicity, which affects the daily timing of sleep and feeding. These results suggest that distinct populations of photoreceptor cells are required for maintaining circadian rhythms in *Drosophila.*

898C Neuronal E93 Regulates Metabolic Homeostasis *Cecilia Yip*[1], Steven Wyler[1], Syann Lee[1], Adrian Rothenfluh[2], Young-Jai You[1], Joel Elmquist[1] 1) University of Texas Southwestern Medical Center, Dallas, TX ; 2) University of Utah, Salt Lake City, UT

The central nervous system integrates environmental and internal cues to drive physiological and behavioral responses to control energy homeostasis. E93 plays an essential role during metamorphosis, yet its neuronal function in adults has not been fully understood.

To determine the central role of E93, we knocked down *E93* expression using nSyb-Gal4 (nSyb>E93), a pan-neuronal driver. Unlike whole-body knockout flies, which cannot progress to eclosion, the nSyb>E93 flies became adults. The nSyb>E93 adults are obese and hyperphagic, with increased energy stores, revealing a novel role of E93 in metabolism. Assessments of whole-body glucose, glycogen, and triglyceride levels indicated a sexual dimorphism in their fuel stores, with males having increased triglyceride stores while virgin females show increased glycogen stores. In order to identify the sites of *E93* action, we targeted E93 RNAi in subsets of neurons using 17 Gal4 lines. We found that *E93* knockdown specifically in myoinhibitory peptide (MIP) producing neurons and GABA-ergic neurons mimics the phenotypes of nSyb>E93. 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 (*EcR*), the known upstream signaling molecule of *E93* specifically in MIP neurons phenocopies the E93 phenotypes, suggesting that steroid hormone signaling to E93 in MIP neurons regulates metabolism. Metabolic dysregulation is often associated with abnormal circadian rhythm. Indeed, nSyb>E93 exhibits a disrupted circadian rhythm; both males and females show reduced activity during the day and decreased day time period.

Together, our study reveals an important neuronal function for E93 in controlling metabolism and provides an insight into how a transcriptional network downstream of a steroid hormone regulates metabolism and circadian rhythm.

899A Adaptive variation in taste detection of carboxylic acids *Manali Dey*[1], Anupama Dahanukar[1,2] 1) Interdepartmental Neuroscience Program, UCR, Riverside; 2) Department of Molecular, Cell and Systems Biology, UCR, Riverside

Comparative studies between generalist and specialist species enable us to learn how adaptations for a particular environment may lead to speciation. *Drosophila sechellia*, endemic to the Seychelles, is an obligate specialist that almost exclusively uses the fruit of *Morinda citrifolia* (noni) for feeding and oviposition. By contrast, its generalist relatives *D. melanogaster* and *D. simulans* take advantage of overripe fruits but avoid noni fruits, which are rich in short to medium chain fatty acids (FA) that are toxic for them. *D. sechellia*, however, are attracted to noni and are able to metabolize the fatty acids. To understand the contribution of the taste system in behavioral adaptation of *D. sechellia*, we undertook a comparative study of gustatory function in the three related species. In two independent behavior assays *D. sechellia* showed a feeding preference for noni acids, whereas the generalist species showed aversion. Genetic and surgical ablation experiments uncovered a contribution of olfaction in stimulating feeding preference for lower concentrations of noni acids, but largely olfaction-independent behavioral responses at the higher end of the concentration range. A systematic comparison of cellular responses with extracellular tip recordings identified two differences in *D. sechellia* that may facilitate their interaction with noni fruit – first, the responses of their deterrent (bitter-sensing) neurons to noni acids are weaker, and second, their appetitive (sugar-sensing) neurons are not strongly inhibited by noni acids. Further, *D. simulans* appears to exhibit phenotypic parameters that are like those of *D. melanogaster* in some cases, and those intermediate between *D. melanogaster* and *D. sechellia* in other cases, suggesting that multiple genetic changes may account for gustatory differences between *D. melanogaster* and *D.*

sechellia. Ongoing studies are directed towards understanding the molecular basis of variation in *Morinda* acid taste between the three *Drosophila* species. Preliminary results suggest the involvement of at least three molecularly distinct pathways involving ionotropic receptors, gustatory receptors, and odorant-binding proteins. We are testing evolutionary roles of candidate genes by manipulating function in both *D. melanogaster* and *D. sechellia*.

900B Functional Genetic Screen to Identify Interneurons Governing Behaviorally Distinct Aspects of *Drosophila* Flight Motor Programs *Sydney Shea*[1], Abby Eisold[1], Lazarina Butkovich[2], Jasper Maniates-Selvin[3], Wei-Chung Lee[3], Matthew Clark[1], Michael Dickinson[2] 1) Bucknell University, Lewisburg, PA; 2) Caltech, Pasadena, CA; 3) Boston Children›s Hospital, Harvard Medical School, MA

Muscles provide the force necessary to generate movements that drive locomotor behaviors. Muscles innervated by single motor neurons comprise synergistic motor units that can be recruited to perform a given task. Drosophila use only a dozen pairs of flight steering motor units to regulate wing motion during both quick maneuvers and slow compensatory reflexes. Steering muscles can be functionally grouped according to their size and patterns of activity. Small tonically active muscles allow the fly to steer straight via subtle adjustments in the phase of firing relative to wing strokes. Large phasically active muscles allow the fly to quickly alter heading via rapid changes in muscle activity. Although features of these motor neurons and muscles are relatively well characterized, the neural network of interneurons controlling them is poorly understood. To study the relationship between neural circuitry and flight, we performed an optogenetic screen of Split Gal4 drivers specific to ventral nerve cord interneurons (VNC INs) and recorded changes in stroke amplitude and wingbeat frequency. Results showed that activation of VNC INs had a variety of effects compared to controls, suggesting that dedicated groups govern specific wing motions. Using the MultiColor FlpOut method we have begun matching morphological features of individual behaviorally relevant neurons to those reconstructed via comprehensive connectomic mapping. Future functional studies will determine how these various interneurons alter the activity of specific steering motor units and thus alter wing motion.

901C The Effect of Cannabidiol on Central Nervous System Development and Function using *Drosophila* as a Model System *Cameron Lowery*, Annika Fischer, Parag Bhatt, Sandra Leal Harris-Stowe State University

Cannabidiol, or (CBD), is a non-psychoactive cannabinoid found in *Cannabis sativa*. While cannabidiol is used by individuals of all ages, little is known about its short and long-term neurological impact on the central nervous system (CNS) or function and development. For this reason, we sought to determine whether CBD oil affects one or more basic behavioral outcomes of the CNS using *Drosophila* as a model system. Several major neurotransmitter systems of *Drosophila* share high conservation with mammalian systems. We assayed three distinct behaviors of Oregon-R wild-type, third-instar larvae under acute (24-hour) and chronic (48-hour) CBD exposure. These behaviors included body wall contractions (BWC), mouth hook contractions (MHC), and the righting reflex (RR). We found that CBD oil differentially affected motor circuits regulating larval MHCs under specific experimental conditions. Acute pretreatment of larvae with 0.5 mg/mL CBD oil significantly reduced larval locomotor behavior but had no significant effect on larval feeding behavior. Conversely, 1.0 mg/mL CBD decreased third-instar larval feeding rates. In comparison to all control group animals, the majority of which righted within 5 seconds or less, the latency of animals righting within the passing score parameters increased for several populations of acute and chronic CBD-treated animals. Since we observed the effects of CBD oil treatment on motor behaviors, we examined neurotransmitter expression levels of tyrosine-hydroxylase (TH) in larval brains exposed to 3.0 mg/mL of CBD. There was no change in the expression level of TH. However, preliminary data suggest an increase in glial cell populations in the brainstem. In conclusion, these studies confirm that CBD is affecting CNS function and development, using a simple model system.

902A *Drosophila* larval burrowing: a parasitoid avoidance behavior? *Meagan Ash*, Todd Schlenke University of Arizona, Department of Entomology, Tucson, AZ

Drosophila melanogaster is a widely used model organism to uncover molecular mechanisms regulating natural behaviors. In the wild, fly larvae experience high rates of attack from endoparasitoid wasps. These wasps inject their eggs into the body of the insect wherein the wasp egg hatches, and the resulting wasp larva eventually consumes the host tissue. *Drosophila* larvae mount a cellular immune response against infection whereby the wasp eggs are encapsulated, melanized, and killed, which wasps attempt to suppress by injecting venoms along with their eggs. *Drosophila* larvae also perform defensive behaviors in the presence of wasps to avoid parasitism, such as moving away from wasp odors, rolling to interrupt wasp oviposition, and self-medication with ethanol to increase their toxicity to the wasp larvae inside them. Here, we describe a new larval defensive behavior in which more larvae burrow into the food substrate in the presence of wasps. We investigate the causes and adaptive value of this behavior, as well as the neurobiological mechanisms underlying it.

903B Characterization of *Drosophila* sugar receptors *LINNI JIN*[1], Seungyun Yu[2], Jae Young Kwon[2], Seok Jun Moon[1] 1) Department of Oral Biology, BK 21 FOUR Project, Yonsei University College of Dentistry, Seoul, Korea; 2) Department of Biological Sciences, Sungkyunkwan University, South Korea

Taste allows animals to discriminate nutritious food from toxic substances. *Drosophila* gustatory receptors (GRs) for aversive chemicals are relatively well characterized; complexes consist of three or four GRs for detecting bitter chemicals. Sweet gustatory receptor neurons (GRNs) express eight closely related GRs: Gr5a, Gr61a, Gr64a, Gr64b, Gr64c, Gr64d, Gr64e, and Gr64f. However, little is known regarding the molecular basics of how sweet GRs detect sugars. Loss of function of Gr64 cluster GRs causes distinct phenotypes, presumably due to the polycistronic expression of mRNA. In addition, findings on ectopic expression of sugar receptors are contradictory to previous studies. To elucidate the function of sugar GRs, we employed the GAL4/UAS overexpression system to overexpress one or a combination of Gr64 genes in the Gr64 null deletion mutant to examine the function of the Gr64 genes. However, this approach also resulted in results inconsistent with the loss of individual Gr64 genes. To overcome the potential artifacts of overexpression of sweet GRs, we generated transgenes to express individual Gr64 cluster genes under the control of the 5' regulatory element of the Gr5a gene, to mimic the endogenous expression of each Gr64 gene. We will present our progress on exploring the necessity and sufficiency of sweet GRs.

904C Meeting a threat of the Anthropocene: Robust taste avoidance of metal ions *Shuke Xiao*, Lisa Baik, John Carlson Department of MCDB, Yale University, New Haven, CT

The Anthropocene era poses a critical challenge for all organisms: they must cope with new threats at a faster rate than ever before. These threats include toxic chemical compounds released into the environment by human activities. Here, we examine high concentrations of heavy metal ions as an example of anthropogenic stressors. We find that different subsets of taste receptors contribute to avoidance behaviors towards eight metal ions when present at high concentrations that flies experienced rarely if ever until the Anthropocene. We analyze feeding and oviposition avoidance behaviors, and we identify taste organs, neurons, and receptors that contribute to the avoidance of metals. We find that metals activate some taste neurons and inhibit others. Receptor mutations have different effects on different avoidance behaviors. Some responses to metals are conserved across diverse dipteran species. Our results suggest mechanisms that may be essential to insects as they face challenges from environmental changes in the Anthropocene.

905A How acetic acid alters interactions of parasitoids with their *Drosophila melanogaster* hosts *Kayla Reddy*[1], Corinne Stouthamer[2], Todd Schlenke[1] 1) University of Arizona; 2) University of Georgia

Drosophila melanogaster, the common fruit fly, is frequently infected by parasitic wasps in nature. Flies have a cellular immune response to encapsulate and kill wasp eggs, but this can be suppressed by injected wasp venom. Flies also display defensive behaviors to prevent infection or cure themselves once infected. For example, it was previously shown that parasitized fly larvae, which live in rotting fruits, medicate themselves by consuming more alcohol. Acetic acid is another potentially toxic product of fermentation sought out by *D. melanogaster*. Here, we test whether acetic acid is beneficial to flies by affecting wasp parasitism. We performed infections on *D*. *melanogaster* using two parasitic wasp species, a generalist and a specialist, and measured parasitism rates in acetic acid, parasitism rates after long term exposure to acetic acid, fly and wasp tolerance of acetic acid, and fly substrate choice with various levels of acetic acid. Our data indicate that acetic acid can protect fly larvae from infection in some scenarios.

906B Dissecting the subcellular mechanisms of signal processing in the Drosophila visual system *Michelle Pang*, Thomas Clandinin Stanford University

Nervous systems process sensory information to extract relevant features from complex environments. While sensory processing algorithms are well-studied, their underlying circuit and molecular implementations remain poorly understood. How do neurons shape signal processing as they transform information from neurotransmitter input, to membrane voltage, to calcium influx, and finally to neurotransmitter release? How do these processes differ among cell types, and what molecular mechanisms drive those differences?
The Drosophila visual system is a highly tractable model for dissecting neural circuits at a subcellular level. Interneurons in the early visual system are genetically accessible, and their synaptic wiring diagram and gene expression profiles are well-characterized. Additionally, many studies have characterized their visual responses and their impact on visually-evoked behavior.
We have assembled a panel of genetically-encoded indicators of voltage, calcium, and neurotransmitter release for in vivo two-photon imaging in order to trace how visual information is transformed as it passes through neurons and across synapses. We display light and dark flash stimuli at multiple contrasts to probe the linearity and kinetics of these subcellular neural responses. Our lab previously showed that voltage and calcium sensors can be used to study visual processing with subcellular resolution, and we have now added an ultrafast calcium sensor and neurotransmitter sensors to our imaging toolkit. We are using these indicators in conjunction with pharmacological and cell type-specific genetic manipulations of ion channels and of neurotransmission to investigate how visual interneurons relay and transform information, as well as what classes of genes regulate signal processing in different neural cell types. By diversifying the types of observations we can make within neurons and synapses, we will gain novel insight into not only how Drosophila visual interneurons participate in signal processing, but also how central nervous system interneurons of different cell types function more broadly.

907C The circuit basis of operant self-administration for ethanol in *Drosophila Melanogaster* *John Hernandez*, Eve Glenn, Nicholas Mei, Reza Azanchi, Karla Kaun Brown University

Alcohol research has largely been focused on understanding the neural mechanisms underlying excessive or compulsive alcohol intake. However, humans display a wide-variety of drinking behaviors with some escalating alcohol consumption over time while others moderately drink or even abstain. Investigating the circuits and neural dynamics underlying this individual variation is critical for developing more effective treatments for alcohol use and abuse disorders. In *Drosophila melanogaster*, the neural circuits required for encoding valence include identifiable connections, genetic and/or biochemical profiles and characterized temporal changes underlying learning, making flies an ideal model for investigating escalation of ethanol self-administration. We developed a 3-day operant paradigm to evaluate the spectrum of behaviors associated with self-administration of a pharmacologically relevant dose of volatilized ethanol and compared these responses to an ethologically relevant ethanol odor. Similar to mammals, individual variation in self-administration behavior for a pharmacologically relevant dose of ethanol occurs with consecutive training sessions. Approximately 35% of flies escalate self-administration whereas 61% of flies remain stable and 4% of flies decrease self-administration. This contrasts significantly with self-administration of ethologically relevant ethanol odor where 8% flies escalate self-administration. Thermogenetically inactivating a simple two neuron cholinergic and dopaminergic mushroom body circuit altered population ethanol preference to decrease and increase ethanol self-administration, respectively. Our data provides the behavioral and neuroanatomical groundwork to subsequently investigate variability in physiology of identified circuits contributing to alcohol use disorder.

908A A toolkit to investigate subtype-specific functions of octopaminergic neurons on fly behavior *Aundrea Koger*, Kenichi Ishii, Kenta Asahina Salk Institute for Biological Studies, La Jolla, CA

All animals must process and respond accordingly to both internal and external stimuli. Modifying behavior is an essential strategy animals employ to respond to a changing environment. Octopamine(OA)–the insect equivalent of norepinephrine–functions as a neurotransmitter, neurohormone, and neuromodulator involved in modulating various behaviors based on sensory input and internal states. The relationship between OA and aggression has been well studied in insects and other arthropods. Because of its expansive genetic toolkit, Drosophila is one of the most useful model organisms for exploring the mechanisms employed by OA neurons to influence aggression. Past studies using Drosophila have demonstrated OA's role in modulating aggression based on various sensory inputs, such as sleep, gut microbiome health, and social experience. Importantly, OA neurons in Drosophila are known to consist of up to 27 anatomically distinct subtypes. Aside from a few examples, it remains in question whether each subtype modulates a specific behavioral process (including context-dependent modulation of aggressive behavior), and if so, which of these OA neurons is responsible for each process. Genetic reagents (such as GAL4 lines) that label specific subtypes of OA neurons will help advance our understanding of the circuits driving octopaminergic modulation of behavior. Currently, only a few OA subtypes are genetically accessible. To investigate the functions of the remaining OA neuronal subtypes, we are isolating split GAL4 lines that label specific subtypes of OA neurons in the central brain. After using immunohistochemistry to visualize the labeling patterns of 367 combinations of split GAL4 Drosophila lines, we have so far found 24 candidates that partially or completely isolate several octopaminergic cell types. Our new genetic reagents will enable further investigation of not only OA's role in aggression circuitry but also cell-type specific control of other behaviors modulated by OA. With more refined access to the circuits underpinning behavior modulation, we can achieve a better understanding of how animals process and respond to the barrage of stimuli they constantly encounter.

909B An Octopaminergic Circuit in Egg Laying *Ethan Rohrbach*, Sonali Deshpande, David Krantz University of California, Los Angeles

Aminergic neurons modulate cellular mechanisms involved in almost every human behavior. Norepinephrine in particular has been shown to modulate physiological processes ranging from heart rate to ovulation. Work from our lab and others has shown that the *Drosophila* equivalent to norepinephrine, octopamine (OA), also has a role in fly ovulation. Processes such as follicle cell rupture in the ovaries, muscle contraction in the oviduct, and sperm storage in the spermathecae have all been shown to be regulated by OA signaling. The specific neurons, receptors, and cellular mechanisms underlying such signaling, however, remain mostly unknown. OA signals to the *Drosophila* reproductive system from a central cluster of OA neurons in the abdominal ganglion, and both alpha and beta types of OA receptors, which are analogous to the alpha and beta types of mammalian adrenergic receptors, are expressed throughout the reproductive organs. Our lab has developed a set of novel preparations and assays for the optogenetic interrogation of this egg laying circuit. We show that stimulation of the OA cluster innervating the reproductive system produces rhythmic contractions of the lateral oviduct muscle. Our work also suggests that the circuit pathway facilitating this action is indirect, including OA signaling to other intermediate neurons rather than directly to the oviduct muscle, which lacks OA receptor expression. This indirect pathway includes a small group of neurons that express insulin-like peptide 7 (ILP7) and a variety of different neurotransmitters/peptides. OA-driven contractions of the lateral oviducts may therefore incorporate contributions from multiple different types of neurotransmission. By mapping the coexpression of other neurotransmitters and receptors in this circuit, such as glutamate and proctolin, and comparing optogenetically-

driven behaviors to those of single-neurotransmitter/peptide perfusions, we aim to define the specific presynaptic and postsynaptic components of this indirect pathway in the OA egg laying circuit. We hypothesize that OA signaling in the egg laying circuit has multiple distinct roles governed by both direct and indirect pathways as well as differences in which OA receptors are expressed in each pathway. This work will help establish a model that can be used to uncover how aminergic neurons coordinate within a single central cluster to regulate multiple different behaviors.

910C Parallel processing of polarized skylight from the optic lobes towards the central brain *Juliane Uhlhorn*[1], Emil Kind[1], Gizem Sancer[2], Thomas Mathejczyk[1], Mathias Wernet[1] 1) Freie Universitaet Berlin, Berlin; 2) Yale University, New Haven, CT

Many animals rely on polarized UV skylight for orientation and navigation. The underlying neuronal circuitry for mediating this behavior in many insects, including *Drosophila melanogaster*, is formed by the Anterior Visual Pathway (AVP). Specialized photoreceptors in the dorsal periphery of the eye, the so-called dorsal rim area (DRA), detect polarized skylight. Additional cell types then process the information from within the optic lobes, before conveying it to a visual glomerulus – the anterior optic tubercle (AOTU) - towards the central complex. The retinotopic information encoded by the angle of polarization (AoP) is conserved throughout this pathway.
Of particular importance are visual projection neurons connecting the medulla neuropil to the central brain. To understand these parallel pathways, we characterize the morphological similarities and/or differences between subtypes using light microscopic techniques such as MultiColor FlpOut (MCFO). Additionally, using GRASP and connectomic EM reconstruction, we investigate potential differences in connectivity. Finally, functional approaches like calcium imaging and quantitative behavior assays are used. Although the overall retinotopic representation is conserved in most subtypes, we find distinct functional differences between them. Hence, simple redundant roles for these parallel channels seem unlikely. We propose a role for these cell types in processing specific navigational cues.

911A Characterization of the mode of transmission of ethanol resistance to progeny of repeatedly intoxicated parental flies Michelle Bonilla, Jocelyn Coreas, Merna Massoud, Antony Ibrahim, *Mariano Loza-Coll* California State University, Northridge, Los Angeles, CA

Characterization of the mode of transmission of ethanol resistance to progeny of repeatedly intoxicated parental fliesThere has been a 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 diverse species, it is still unclear what genetic pathways may connect acquired traits that operate primarily in metabolic, physiologic or nervous system levels with the necessary epigenetic modifications in the germline for transmission to take place. While classic model organisms, such D. melanogaster and C. elegans are prime candidates to dissect conserved aspects of the genetic and epigenetic underpinnings of acquired trait inheritance, little is known about their capacity to transmit acquired drug tolerance to their progeny.

Numerous previous studies over the last 20 years have demonstrated that fruit flies can develop tolerance to ethanol when repeatedly exposed to the drug. We have recently reported that parental flies that are intoxicated multiple times (once a day, for 10 minutes, over a 2 weeks period) give rise to progeny that is significantly more resistant to the sedative effects of ethanol. We observed that parental flies need to be exposed multiple times before transmission of resistance to progeny can be observed, that there is residual transmission of tolerance to the F2 and that the ability of females to transmit tolerance to their F1 progeny lasts several days after their last intoxicating exposure to ethanol. We have since more carefully explored whether the transmission of resistance to progeny of repeatedly intoxicated flies is matrilineal, patrilineal or both, which will delineate future research efforts aimed at elucidating the genetic and molecular bases of the transmission. Overall, our findings present D. melanogaster as a suitable experimental model for forward and reverse genetics approaches to investigate the pathways connecting repeated exposures of an adult animal to ethanol and the necessary germline modifications that underlie the transmission of increased resistance in their progeny to the drug.

912V Pre-copulatory reproductive behaviours are preserved in Drosophila melanogaster infected with bacteria. *Saloni Rose*[1], Esteban Beckwith[2,3], Charlotte Burmester[1], Robin May[4], Marc Dionne[2], Carolina Rezaval[1] 1) University of Birmingham; 2) MRC Centre for Molecular Bacteriology and Infection and Department of Life Sciences, Imperial College London, London SW7 2AZ, United Kingdom; 3) Instituto de Fisiología, Biología Molecular y Neurociencias (IFIBYNE), UBA-CONICET, Buenos Aires, Argentina; 4) Institute of Microbiology & Infection, University of Birmingham, Birmingham B15 2TT, United Kingdom

The activation of the immune system upon infection exerts a huge energetic demand on an individual, decreasing available resources for other vital processes, such as reproduction. However, the factors that determine the trade-off between defensive and reproductive traits remain poorly understood. Here, we exploit the experimental malleability of

the fruit fly Drosophila melanogaster to systematically assess the impact of immune system activation on pre-copulatory reproductive behaviour. Contrary to our expectations, we found that male flies undergoing an immune activation continue to display high levels of courtship and mating success. Similarly, immune-challenged female flies remain highly sexually receptive. By combining behavioural paradigms, a diverse panel of pathogens and genetic strategies to induce both arms of the fly immune system, we show that pre-copulatory reproductive behaviours are preserved in infected flies, despite the huge metabolic cost of infection.

913V Identification of individual essential amino acid sensors in *Drosophila* Jinhyeong Lee, *Jong-Hoon Won*, Boram Kim, Greg Suh KAIST

A balanced intake of macronutrients such as proteins, carbohydrates and fats is essential for the organism, and insufficient protein intake leads to several diseases, including kwashiorkor. Protein-deficient animals have a dietary behavior which is selecting food sources that contain essential amino acids (EAAs) rather than non-essential amino acids. Recently, a neuropeptide called CNMamide (CNMa) was found in *Drosophila melanogaster* to be highly expressed in enterocytes of the midgut during protein depletion to modulate EAA-specific selective dietary behavior. *CNMa* expression in these enterocytes is up-regulated in response to protein depletion through the GCN2-ATF4 and TOR-MITF pathways, each of which trigger a compensatory appetite for EAA (Kim, et al. 2021, *Nature*). Remarkably, flies fed a holidic diet lacking a single L-EAA had enhanced *CNMa* expression, suggesting the presence of a molecular sensor that recognizes each L-EAA. We have carried out RNAi screening to identify candidate molecules that sense or detect each EAA and are currently characterizing these putative sensors using molecular, genetic and biochemical approaches.

Kim, B., Kanai, M.I., Oh, Y. *et al.* Response of the microbiome–gut–brain axis in *Drosophila* to amino acid deficit. *Nature* **593,** 570–574 (2021). https://doi.org/10.1038/s41586-021-03522-2

914V *Neuroligin3* and dopamine are required for a response to social isolation, but recovery is complex and sex-specific. *Ryley T Yost*, Branden Walshe-Roussel, Anne F Simon University of Western Ontario

Social isolation causes profound changes in social behaviour in a variety of species including humans, monkeys, mice, bees, and vinegar flies. However, the genetic and molecular mechanisms modulating behavioural responses to both social isolation and social recovery remain to be elucidated. Here, we quantified the behavioural response of vinegar flies to social isolation using the flies' social space preference. Flies with a loss of function of *neuroligin3* (ortholog of autism-related *neuroligin* genes) with known increased social space in socially enriched environment, responded similarly to their genetically matched control to both social isolation and recovery. We next used a *UAS-TH*-RNAi driver in all neurons to investigate the role of dopamine, another known modulator of a response to social isolation. We show that dopamine is important for a response to social isolation and recovery in males but not in females. Furthermore, only in males, dopamine levels are reduced after isolation and are not recovered after group housing. Finally, in socially enriched flies with a loss of function of *neuroligin3*, dopamine levels are reduced in males, but not in females. We will share a model to explain how dopamine and *neuroligin3* are involved in the behavioural response to social isolation and its recovery in a dynamic and sex-specific manner.

915V Behavioral Characterization of *tecu* Mutants *Laura Alejandra Lujano Perez*[1], Juan Rafael Riesgo Escovar[2] 1) Maestría en Ciencias (Neurobiología), Universidad Nacional Autónoma de México; 2) Instituto de Neurobiología, Universidad Nacional Autónoma de México

We isolated mutations in a gene that we named *tecuzitécatl* (*tecu*), that codes for a phospholipase A_2 enzyme narrowly expressed in a group of Kenyon cells, which are the mushroom bodies intrinsic neurons in the brain of *Drosophila melanogaster*. Here we show that mutations in this gene lead to faulty behavioral responses in visual paradigms. We directly compared responses of control (*yw*) and two mutant *tecu* strains (*tecu*^{1} and *tecu*^{2}), because all of them share the same genetic background. We first performed a larval phototaxis assay. Results show that they present significantly different responses (migrate more towards the light). We also performed an adult countercurrent assay, where they have a deficient response to light (have significantly less positive phototaxis). Taken together, these results show that *tecu* has an important role processing visual responses in *Drosophila melanogaster*.

916V Local 5-HT signals bi-directionally modulate the coincidence time window of associative learning *Xuelin Li*, Jianzhi Zeng, Zimo Zhangren, Yulong Li Peking University, School of Life Sciences

Temporal coincidence between a conditioned stimulus (CS) and an unconditioned stimulus (US) is an essential component in Pavlovian associative learning. Despite its ubiquitous presence, it remains unknown whether the coincidence time-window can be regulated. Here we assign this function in Drosophila to the dorsal paired medial (DPM) neuron, which innervates the mushroom body (MB) and releases 5-HT compartmentally in response to both an odorant (the CS) and electric shock (the US). The DPM neuron is activated by local acetylcholine (ACh) release from MB

Kenyon cells through nicotinic ACh receptors, meanwhile releases 5-HT to reduce ACh release via the 5-HT1a receptor. Suppressing or promoting the 5-HT release from DPMs bi-directionally regulates the coincidence time-window for odor-shock pairing, which gates olfactory learning and synaptic plasticity changes in the MB. This mechanism provides a model for studying how an organism times the temporal continuity of environmental events and learns the underlying causal relationship.

917V Spying on the dynamics of octopamine by genetically-encoded GRAB$_{OA}$ sensor in *Drosophila* *Mingyue Lv*[1,2], Ruyi Cai[1,2], Huan Wang[1,2], Yulong Li[1,2,3,4] 1) State Key Laboratory of Membrane Biology, Peking University School of Life Sciences, Beijing, China; 2) PKU-IDG/McGovern Institute for Brain Research, Beijing, China; 3) Peking-Tsinghua Center for Life Sciences, Academy for Advanced Interdisciplinary Studies, Peking University, Beijing, China; 4) Chinese Institute for Brain Research, Beijing, China

Small molecular neurotransmitters are essential for neuronal communication in the nervous system. Octopamine (OA), the analog of norepinephrine in invertebrates, is an important monoamine neurotransmitter involved in many physiological events of insects, such as the aggression, flight, ovulation, learning and memory. Although much is known regarding the properties of OA, its spatial and temporal dynamics are poorly understood at the *in vivo* level due to the limited detecting methods. Here, to directly visualize the release of OA, we developed a genetically encoded G-protein-coupled receptor (GPCR) – activation – based OA (GRAB$_{OA}$) sensor. In response to extracellular OA, GRAB$_{OA}$ shows large fluorescent increase (~500% DF/F0), high selectivity and fast kinetics to micromolar OA. In living flies, we observed GRAB$_{OA}$ expressed in the Kenyon cells (KCs) responses to electrical stimulation in a dose dependent pattern. For optogenetic activation of octopaminergic neurons, we detected homogenous fluorescent increase across mushroom body, which is the center for *Drosophila* olfactory associative learning. Moreover, GRAB$_{OA}$ was able to reports physiological stimulation including odorant and abdomen shock induced OA release. Thus, GRAB$_{OA2m}$ enables spatiotemporally precise measurements of OA dynamics *in vivo* in *Drosophila*, which provides the possibility to explore the role of OA in different interesting physiological process in invertebrates.

918V Two Individually Identified Paired Dopamine Neurons Signal Taste Punishment in Larval *Drosophila* Denise Weber[1], Katrin Vogt[2], *Andreas Thum*[1] 1) Institute of Biology; Department of Genetics, University of Leipzig, Germany; 2) Department of Neurobiology, University of Konstanz, Germany

Dopaminergic neurons (DANs) perform multiple tasks in the brain. Among other things, DANs mediate teaching signals such as information about rewards and punishments throughout the animal kingdom. They serve to evaluate sensory input, store the resulting associations as memory, and continuously update them according to relevance and reliability. Therefore, to better understand the functioning of the dopaminergic system, it is crucial to know what specific roles are mediated by each DAN. To this end, we are studying *Drosophila* larvae, whose brains consist of only about 12,000 neurons, of which only about 1% are DANs.

Only eight larval DANs presynaptically project to the mushroom body, a brain region in insects that is of central importance to associative olfactory learning. These eight DANs in turn anatomically subdivide into four cells of the primary protocerebral anterior medial cluster (pPAM) and four cells of the dorsolateral 1 cluster (DL1). As we have shown in previous studies, the activity of the pPAM DANs encodes the internal gustatory sugar reward signal. In this study, we investigate the four DANs of the DL1 cluster in terms of their cellular function with respect to gustatory teaching signals. We discover that two DANs (DAN-f1 and DAN-g1) innervating two specific compartments of the vertical lobe of the mushroom body are in combination acutely necessary for learning of odor-high salt punishment, but dispensable for appetitive learning and innate behavior toward the applied odors and salt. Optogenetical activation of DAN-f1 and DAN-g1 neurons in the larval brain is sufficient to encode punishment. The functional specificity of DAN-f1 and DAN-g1 within the DL1 cluster and towards the PAM cluster is further confirmed by synaptic reconstruction of sensory and DAN input neurons. Thus, the DL1 neurons convey a punishment teaching signal.

In summary, this indicates that a cellular division of labor of larval DANs exists with respect to the transmission of dopaminergic reward (pPAM cluster) and punishment (DL1 cluster) signals. Since the organizing principle of larval DANs is the same as that of its adult counterpart and that of the mammalian basal ganglion, it is possible that there are only a limited number of efficient neural circuit solutions to solve complex cognitive and behavioral problems in nature.

919V Single cell transcriptomic analysis of homologous courtship song neurons between species *Justin Walsh*[1], Johaer Jilani[1], Lihua Wang[2], Yun Ding[1] 1) University of Pennsylvania; 2) Janelia Research Campus of the Howard Hughes Medical Institute

Understanding the evolution of complex traits, including behavior, is a main goal of evolutionary biology. Behavioral diversity across species is expected to be the result of evolutionary changes in neural morphology and function. In turn, a neuron's morphology and function is the 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 song, wing vibrations generated by a male while courting a female, displays a wide range of diversity across species. For example, *D. melanogaster* males sing two types of song, pulse and sine, by extending and vibrating one wing. In contrast, *D. yakuba* males do not sing sine song but instead sing a song called clack by vibrating both wings without obvious extension. A male-specific single bilateral pair of neurons named pIP10 is necessary for pulse song in *D. melanogaster* but clack song in *D. yakuba*. In both species, we genetically labeled pIP10 neurons with a GFP marker and manually isolated them for transcriptomic profiling. We found that many genes are expressed in a species-specific or -biased manner including some with known neural or behavioral functions. Next, in *D. melanogaster* we used pIP10-specific RNAi to knock down genes upregulated in *D. melanogaster* pIP10 neurons relative to *D. yakuba* pIP10 neurons and found that some of our knockdowns resulted in mutant *D. melanogaster* song phenotypes. Overall, our study is one of the first to compare gene expression in homologous neurons across species and offers a novel perspective on how evolution has shaped gene expression patterns to result in functional adaptation across species.

920V Investigating the Role of SIFamide in the Effects of Food Deprivation on Female Reproductive Drive *Attilio Ceretti*, Jill Schneider Lehigh University

Adaptation requires differential reproductive success, but reproductive processes are energetically expensive and can compromise the chances of survival under energetic challenges. Thus, many species appear to have evolved mechanisms that inhibit reproduction when energy is scarce. For example, in a genetically heterogenous population of *Drosophila melanogaster* derived from an orchard in New Jersey, we found that 36-48 hours of food deprivation in females significantly decreased the incidence of copulation with fed males, whereas food deprivation in males had no effect on courtship or copulation attempts with fed females. One signal that could be mediating these results is the evolutionarily conserved neuropeptide, SIFamide, the insect ortholog of the mammalian peptidergic/hormonal signal RFamide-related peptide-3, which mediates the effects of energy on reproduction in hamsters. To test whether this peptide mediates the effects of energy on reproduction, RNAi towards SIFamide was expressed in SIFamide neurons. Wildtype control females decrease their mating rate and increase their latency to copulate following only 24 hours of food deprivation, while females that express RNAi toward SIFamide do not. Knockdown of SIFamide blocks the effects of food deprivation on female reproduction, consistent with the idea that SIFamide expression is important for the inhibition of reproduction when energy is scarce. Our results provide a potential neurological basis for an ancestral system that mediates tradeoffs in food intake and reproduction.

921V Chronic caffeine teatment disrupts circadian rhythm in *Drosophila* *Aishwarya Segu*, Nisha Kannan Indian Institute of Science Education and Research, Thiruvananthapuram

The circadian clock governs the timing of sleep-wake cycles as well as of other behavioural, physiological and metabolic processes. While the endogenous circadian clock mediates the timing of sleep, homeostatic mechanisms modulate the amount and depth of sleep. Evidence from previous studies showed that caffeine intake promotes wakefulness, whereas adult-stage specific caffeine treatment not only suppresses sleep but also delays the phase of circadian rhythm in Drosophila. In humans, caffeine is consumed on a daily basis and hence it is important to understand the effect of prolonged intake of caffeine on circadian and homeostatic regulation of sleep. In the present study we examined the differential effect of acute and chronic caffeine treatment on sleep ontogeny as well as on circadian and homeostatic regulation of sleep in *Drosophila*. The results of our study showed that acute caffeine treatment reduces both day and night sleep in mature flies whereas it reduced only the day sleep in young flies. Chronic caffeine treatment did not exert any significant effect on sleep in young flies. On the other hand, it delayed the timing of sleep in mature flies and in addition these flies reduced the morning and evening anticipatory activity at higher caffeine concentration under 12 hour: 12 hour light: dark cycles. Apart from these it also changed the overt rhythm of these flies. These flies exhibited either a longer free running period or arrhythmicity under constant darkness. The results from our study shows that acute treatment of caffeine reduces sleep in flies and does not affect the homeostatic sleep whereas prolonged caffeine treatment affects the overt rhythm of the flies including the eclosion rhythm.

922V Aggression in *Hieroglyphus banian* (Rice grasshopper) vs. in *Drosophila melanogaster*: A Comparison *Abhilash Kondai*, Sudipta Saraswati University of Hyderabad, Hyderabad, India

While aggression has been reported to be a widely observed behavior across animal species, it has not been systematically studied in many organisms. For example, aggressive behavior in *Drosophila melanogaster* has been well-documented but that is not the case with grasshoppers. We undertook an extensive study of aggressive behavior in a species of rice grasshopper, *Hieroglyphus banian.* Here, we present our findings of aggression in male *Hieroglyphus banian* and compare our findings with published literature of aggression in male *Drosophila melanogaster.* We found that several components of aggressive behavior, such as (i) approach, (ii) holding or grabbing, (iii) low-level fencing or step on, (iv) tussling, and (v) walking away or running away or flying away, are conserved between grasshopper and *Drosophila*. However, we also observed that biting is an aggressive behavior that is exhibited by only grasshopper and is not reported in *Drosophila*. Interestingly, in grasshopper we did not observe some behavioral components of

aggression, such as (i) wing threatening, (ii) lunging, and (iii) boxing, that are reported to be exhibited by *Drosophila*. Our findings and comparative analysis indicate that several components of aggressive behavior are conserved between two insect species of two different kinds—hemimetabolous insect grasshopper and holometabolous insect *Drosophila*. Results from our study will help us further investigate if the aforementioned conserved aspects of aggressive behavior are conserved across a variety of insect species. Moreover, our study also forms the basis for investigations into the functional significance of the conserved vs. non-conserved aspects of aggressive behavior across different insect species.

923V Intestinal CNMa induced by protein deficit affects two distinct pathways in the brain to regulate the preference for protein-rich food *Boram Kim*[1], Seongju Lee[1], Yangkyun Oh[2], Makoto Kanai[2], Won-Jae Lee[3], Greg S. B. Suh[1] 1) Department of Biological Sciences, Korea Advanced Institute of Science and Technology, Daejeon, Republic of Korea; 2) Skirball Institute of Biomolecular Medicine, Department of Cell Biology, New York University Grossman School of Medicine, New York, NY, USA; 3) National Creative Research Initiative Center for Hologenomics and School of Biological Sciences, Seoul National University, Seoul, Republic of Korea

The ability to maintain protein homeostasis is crucial for survival and fitness. While it is known that the taste sensory system recognizes the availability of dietary proteins in the environment, it remains unclear how animal detects and responds to the deficit of amino acid levels in the internal milieu. Essential amino acids (EAAs) cannot be synthesized and must be consumed through diet. CNMamide (CNMa) is released from a specific population of enterocytes in the *Drosophila* gut during EAAs deprivation and causes increased appetite for EAAs (*Kim et al., Nature 2021*). However, the mechanism by which CNMa influences the brain for the promotion of EAAs consumption remains poorly understood. Here, we show that among many regions of the brain that express CNMa receptors, two distinct regions of the brain that affect protein and carbohydrate appetite are activated or inhibited by CNMa. These findings reveal the interplay between the gut and the brain for the regulation of nutrient-specific appetite.

Kim, B., Kanai, M.I., Oh, Y. *et al.* Response of the microbiome–gut–brain axis in *Drosophila* to amino acid deficit. *Nature* **593,** 570–574 (2021). https://doi.org/10.1038/s41586-021-03522-2

924V Molecular and cellular basis of acid taste sensation in *Drosophila* *Ting-Wei Mi*[1], John Mack[1], Christopher Lee[2], Yali Zhang[1,3] 1) Monell Chemical Senses Center, Philadelphia, PA; 2) Department of Biology, University of Pennsylvania, Philadelphia, PA; 3) Department of Physiology, The Diabetes Research Center, University of Pennsylvania, Perelman School of Medicine, Philadelphia, PA

Sour taste is one of the five commonly accepted basic taste modalities, alongside four other fundamental taste: bitter, sweet, salty, and umami. This taste sensation is evoked by acidic foods such as vinegar or certain fruits, and allows organisms to avoid consuming unripe or spoiled, or fermented foods. Species from insects to mammals are attracted by slightly acidic foods, but repulsed to highly acidic foods. However, the molecular and cellular mechanism of how animals distinguish low from high acid foods is still largely unknown. Here, we use the fruit fly (*Drosophila melanogaster*) as a model organism to address this question. Firstly, we show that fruit flies employ two competing taste sensory pathways to discriminate low from high acidity, which trigger attractive or aversive behavior respectively. Secondly, we identify Otopetrin-like a (OtopLa), a member of an evolutionarily conserved gene family, as a proton channel in fruit flies expressed in bipolar type gustatory receptor neurons (GRNs) in the proboscis with central projection patterns in the subesophageal zone (SEZ), which receives taste input from the peripheral taste organs. Loss of *otopla* impairs flies' acid sensation, causing an imbalance in the competing taste pathways leading to an abnormal aversive response to low acid. In summary, our behavioral, anatomical, and electrophysiological data strongly suggest that flies display a competing acid-sensing mechanism to distinguish between high and low acid, and the proton channel OtopLa is required for low acid sensation. This sensing mechanism that may be evolutionarily conserved between insects and mammals, given the similarity in their taste preferences for acid and homologous functions of Otop genes.

925V Gastric mechanosensation and the peptidergic sugar sensing regulate the *Drosophila* nutrient sensor *Yangkyun Oh*[1], Jason Lai[1], Soohong Min[2], Huai-Wei Huang[1], Stephen Liberles[2], Hyung Don Ryoo[1], Greg Suh[1,3] 1) Department of Cell Biology, NYU Grossman School of Medicine, New York, NY 10016; 2) Harvard Medical School, Howard Hugh Medical Institute, Department of Cell Biology, Boston, MA 02115; 3) Department of Biological Sciences, Korea Advanced Institute of Science and Technology, Daejeon 34141, Republic of Korea

Nutrient sensors allow animals to identify foods rich in specific nutrients. The *Drosophila* nutrient sensor, DH44 neurons, helps the fly to detect nutritive sugar. This sensor becomes operational during starvation; however, the mechanisms by which DH44 neurons or other nutrient sensors are regulated remain unclear. Here, we identified two satiety signals that inhibit DH44 neurons: 1) Piezo-mediated stomach/crop stretch after food ingestion; 2) Neuromedin/Hugin neurosecretory neurons in the ventral nerve cord (VNC) activated by an increase in the internal glucose levels. A subset of Piezo$^+$ neurons that express DH44 neuropeptide project to the crop. We found that DH44 neuronal activity and food intake were stimulated following a knockdown of piezo in DH44 neurons or silencing of the VNC Hugin neurons, even in fed flies. Together, we propose that these two qualitatively distinct peripheral signals work in concert to regulate the

DH44 nutrient sensor during the fed state.

926V Screening of genes that regulate the maintenance of synapse during aging of *Drosophila melanogaster* *Danielle Moreira*, Jessica Sidisky, Daniel Babcock Department of Biological Sciences, Lehigh University, Bethlehem, PA

The maintenance of synapses is dependent on several coordinated mechanisms, which involve cell-to-cell communication, molecules recycling and degradation. However, the mechanism that controls the synapse maintenance during aging is poorly understood. Here, we investigated the synapse maintenance at the neuromuscular junction (NMJ) of adult *Drosophila melanogaster* lines. Using a flight behavior tester, we evaluated the flight ability of 205 inbred fly lines from the Drosophila Genetic Reference Panel (DGRP), which harbor known SNP and non-SNP variants in or near genes, during aging. To flight test, we collected flies, which were reared on an incubator at 25°C for 1- or 2-days post pupae eclosion, separated by sex and matured them for 3 days and 21 days on an incubator at 29°C. We observed a widely variable flight ability in the DGRP lines from day 3 to day 21 during aging. However, 10 out of 205 DGRP lines had the most expressive decline of the flight ability during aging. To validate these findings, we tested mutant flies for these 10 genes and observed that these genes are prone to cause progressive loss of flight ability. In addition, to identify how these genes cause disruption on tripartite synapse of the neuromuscular junction (NMJ), we used a UAS-Gal4 system to direct the knockdown of those genes on glia (Repo-Gal4), muscle (MHC-Gal4) and motor neuron (BG380-Gal4). Knocking down the 10 genes on muscle and/or motor neurons led to flight ability decline, but one out of the 10 genes caused a progressive defect of the flight behavior directed by the glial driver. To further understand the cellular alterations on synapse at the NMJ, we are characterizing the morphology of the pre- and post-synaptic components. Our data is highlighting the mechanism regulating the synapse maintenance during aging and the progressive degeneration of the synapse cellular components.

927V IFT88 maintains sensory cilia function in *Drosophila melanogaster* Sascha Werner[1], *Pilar Okenve-Ramos*[1], Sihem Zitouni[2], Philip Hehlert[3], Susana Mendonça[5], Anje Sporbert[4], Christian Spalthoff[3], Martin C. Göpfert[3], Swadhin C. Jana[1], Mónica Bettencourt-Dias[1] 1) Instituto Gulbenkian de Ciência, Oeiras, Portugal; 2) Institut de Génétique Humaine (IGH) UMR 9002 CNRS, Montpellier, France; 3) Department of Cellular Neurobiology, University of Göttingen, Germany; 4) Advanced Light Microscopy, Max Delbrück Centrum for Molecular Medicine Berlin in the Helmholtz Associatio, Berlin, Germany; 5) Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, Portugal.

Cilia are involved in a plethora of motility- and sensory-related functions. Ciliary defects cause several ciliopathies, some of which with late onset, suggesting cilia are actively maintained. While much is known about cilia assembly, little is understood about the mechanisms of their maintenance. Given that intraflagellar transport (IFT) is essential for cilium assembly, we investigated the role of one of its main players, IFT88, in ciliary maintenance.
We show that *Dm*IFT88/NOMPB, the Drosophila melanogaster orthologue of IFT88, continues to move along fully formed sensory cilia. We further identify the Drosophila Guanylyl Cyclase 2d (*Dm*Gucy2d/CG34357) and the TRPV channel subunit -Inactive- as *Dm*IFT88 cargoes. In particular, *Dm*IFT88 binds and is important for the localisation of the intracellular part of *Dm*Gucy2d, which is evolutionarily conserved and mutated in several degenerative retina diseases. We find that acute knockdown of *Dm*IFT88 and *Dm*Gucy2d in ciliated neurons of adult flies leads to defects in the maintenance of cilium function, impairing hearing, and gravitaxis behaviour.
Our results show that cilia function requires an active maintenance program and that *Dm*IFT88, and two cargoes, are important for this process. In addition, our work suggests that this maintenance program may be deregulated in degenerative human diseases where Guanylyl Cyclases are mutated.

928V Exploring the functional evolution of odorant receptors in bark beetles using Drosophila empty-neuron system *Jibin Johny*, Ewald Große-Wilde, Blanka Kalinová, Fredrick Schlyter Czech University of Life Sciences Prague

Bark beetles (Coleoptera: Curculionidae: Scolytinae) are a highly diverse subfamily of weevils generally considered as economically highly relevant pests of forests. As a natural part of forest ecosystems, they rely on damaged trees, but can switch to healthy hosts during the 'aggressive' outbreak phase (Biedermann et al., 2019). Bark beetles employ species-specific aggregation pheromones to attract conspecifics to their host trees (predominantly conifers) for the purposes of mating and resource exploitation. Within the Scolytinae, a clear phylogenetic pattern has been observed in pheromone composition; some components are phylogenetically more conserved than others (Symonds and Elgar, 2004; Symonds and Gitau-Clarke, 2016). The differences in pheromone composition are greater between closely related species than with distantly related species. This indicates a rapid 'switching on and switching off' of certain compounds in aggregation pheromone composition (Symonds and Elgar, 2008, 2004). From the evolutionary perspective, we hypothesize a similar level of rapid and heritable switches in olfactory reception at molecular level, *i.e.* in the structure and expression of pheromone-detecting receptors. We therefore investigate the evolution of chemosensory gene families in closely related *Ips* species. Species were selected based on niche level differentiation (syntropy), feeding modes and availability in the Czech Republic (Lindgren and Raffa, 2013). An antennal transcriptome and, more recently, the genome of *I. typographus* have become available (Andersson et al., 2013; Powell et al., 2021) and our research extends this knowledge to other species using both transcriptomes and genomes. For functional analysis we

use a *Drosophila* transgenic expression system (so called “empty neuron”) in conjunction with electrophysiological measurements to characterize receptors by responses (Gonzalez et al., 2016). Overall, this research will provide better understanding of olfactory perception in bark beetles and thereby support development of olfaction-based pest-control strategies.

929V Genetic dissection of physiological properties of local interneurons in the *Drosophila* larval visual circuit *Hsueh-Ling Chen*, Anna Grigsby-Brown, Aidan Dermady, Quan Yuan National Institute of Neurological Disorders and Stroke, NIH, Bethesda, MD

Animals continually modify their behaviors in response to the changing environment. Understanding the molecular and circuit basis of how the nervous system modifies its function to adjust behavioral output is an active topic in the neurobiology field. The *Drosophila* larval visual circuit serves as a suitable system to study this fundamental question. Composed of only twelve photoreceptors (PRs) to detect visual stimuli, one pair of visual local interneurons (VLNs) to modulate the signals, and nine visual projection neurons (VPNs) to transfer the filtered information to higher brain regions, larvae employ a simple visual circuit for a variety of behaviors. Specifically, recent connectome and functional imaging analyses revealed these VLNs, which have been identified as one cholinergic (cha-IOLP) and one glutamatergic (glu-IOLP), synaptically interact with each other as well as receive neuromodulatory input from serotonergic and octopaminergic neurons, suggesting their potential role as plastic components in the circuit for internal or external signals to modulate visual information and impact behavior. Our previous study suggests that excitatory and inhibitory inputs into the VLNs are mediated by glutamatergic and cholinergic signaling. To better understand how visual signals are processed and modulated at the level of VLNs, we aim to evaluate the interactions between these two antagonizing pathways within larval VLNs. First, to identify the neurotransmitter receptors expressed in each VLN, we performed a comprehensive survey on acetylcholine receptors (AChRs) and glutamate receptors (GluRs) using endogenously-tagged GAL4 and LexA lines. Our preliminary results offered an overview of the combinatory expression of nicotinic AChR (nAChR) subunits and muscarinic AChRs (mAChRs) as well as that of ionotropic and metabotropic GluRs in larval VLNs. Second, to validate the screen results, we are performing immunostaining and genetic manipulations, in combination with physiological and behavioral tests. Our results will provide molecular insights into how sensory information is computed and transmitted within a sensory circuit and help us identify specific cellular and molecular components targeted by developmental and activity-dependent regulatory mechanisms.

930V Manipulation of neuron transmission in the mushroom bodies and protocerebral bridge affects social behaviour *Abigail Bechard*, J. Wesley Robinson, Ryley Yost, Anne Simon Western University, London ON, Canada

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 (Nlg-3), 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 both simultaneously. 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 Nlg-3 and the neural circuitry which modulates social space as well as the effects of silencing neuronal transmission in the PB.

931V Understanding the neural circuity of social spacing behaviour through the lens of *Drosophila* Neuroligin 3 *John Robinson*, Abigail Bechard, Ryley Yost, Anne Simon Western University

The neural circuitry underlying *Drosophila* social behaviours has become an increasing field of study. *Drosophila* exhibit social behaviours when in proximity to other individuals and settle at a defined social space. Determination of social spacing could be attributed to many factors such as social experience, genetics, or differential signalling of neural circuitry. So far, we and others have determined that the mushroom bodies (MB) of the *Drosophila* brain are important for social space. As well, synaptic proteins including Rugose a scaffolding protein at the postsynaptic density, Narrow-

abdomen a channel at the presynaptic terminal, and proteins involved with dopamine synthesis and vesicle loading all play a role in social space - highlighting the importance of synaptic functioning on this behaviour. The manipulation of global dopaminergic signalling also alters social space in a sexually dimorphic manner; however, we do not know how dopamine signalling, synaptic proteins, and the MB are all connected to modify fly social spacing. Here, we show that a deficiency of *neuroligin 3 (nlg3),* a gene encoding a post-synaptic protein that regulates transmission at the synapse, modifies social space. Neuroligin 3 is also found to be enriched in the MB, protocerebral bridge, and optic lobes regions of the brain. Hyperactivation and silencing of *fruitless* neurons recapitulates the sexually dimorphic effect we previously observed in fly social spacing, so lastly we targeted a dopaminergic knockdown within the P1 subset of *fruitless* neurons. Our results contribute to understanding the role of specific neural circuitry in modulating the social spacing behaviour of *Drosophila*.

932V Don't want to be all by myself BUT Don't stand so close to me *Anne F Simon*, Ryley T Yost, J Wes Robinson, Abigail T Bechard University of Western Ontario

Before engaging in complex social interactions, flies must determine their preferred social space. Indeed, all motile organisms, from bacteria to humans, including *D. melanogaster*, display a preferred inter-individual (or social) distance that can be affected by genetics, experience and/or the environment.
One of the focus of my lab, is to better understand the neurogenetic underpinnings of 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, and an interplay between those. A sex specific neural circuit is emerging as a modulator of social spacing: it involves dopaminergic signalling, and two major brain structures: the mushroom bodies, and protocerebral bridge.
In addition, the neural bases of social spacing are starting to be elucidated. And several of the players – from neurotransmitters to post-synaptic proteins – are conserved through evolution. At the synaptic level, we have shown that Neurobeachin (an anchor protein) and Neuroligin (a cell adhesion protein) are implicated in social space, as well. Both of those postsynaptic proteins have human homologues that are candidate genes for Autism Spectrum Disorders (ASDs).
In another type of response to social cues, flies strongly avoid the volatile substance dSO emitted by stressed flies. CO_2 has been identified as one of the compounds in dSO, although other unidentified compound(s) are required to elicit the full avoidance of dSO. Because dSO is emitted by stress flies and causes a response from conspecifics, it is considered a social cue. Compared to social space, dSO avoidance requires different sensory modalities such as olfaction. Surprisingly, we found that only a very light stress is enough to elicit a strong dSO avoidance response by the flies, potentially influencing flies in all sorts of behavioural assays performed in typical *Drosophila* research laboratories. Furthermore, elucidating what chemical the flies are avoiding, and whether other species would avoid this chemical could open the door to pest management applications.
I will present the recent progress my research team has made in elucidating the basis underlying the decision-making process to come to around 2-body length away from another fly, and to avoid another one when an alarm signal is perceived.

933V Some Innexin Family Members Are Required for Cold Nociception Responses Mediated by Class III Dendritic Arborization Neurons *Nicolas Nettemeyer*[1], Rachel Barborek[1,2], Maddie Ward[1,3], Susan Halsell[1] 1) James Madison University, Harrisonburg, VA; 2) University of Washington, Seattle, WA; 3) Virginia Commonwealth University School of Medicine, Richmond, VA

The class III dendritic arborization (da) neurons mediate the behavioral response of third instar larvae to noxious cold (Turner *et al.* 2016. Curr Biol. 26:3116). Larvae respond to noxious cold by decreasing their overall length in a manner referred to as cringing. Innexin proteins form gap junctions, and may function in electrical synapsis, development of the nervous center, or tissue morphogenesis. GAL4/UAS RNAi expression coupled with a cold behavioral response assay assessed the possible role of each of the eight *Drosophila* Innexin family members. Expression of *innexin* gene-specific RNAi's was driven specifically in class III da neurons. When possible, more than one RNAi line was studied for each gene. Alteration of the wild-type cold behavioral response was quantified as a loss of the cringe response (non-cringing). Of the eight *innexin* genes, RNAi expression against *innexins 1* (*ogre*), *2*, and *5* significantly inhibited the cold nociception response with both tested RNAi lines. The requirement for Innexin 1 function was confirmed by examining the response in *ogre* complete loss-of-function mutants. *innexins 3, 4 (zero population growth), 6,* and *8 (ShakingB)* showed inconsistent results between their two tested RNAi lines. In order to resolve the ambiguity, behavioral assays are underway to test double-stranded RNAi constructs for each of these genes. Finally, RT-PCR is being conducted to determine which of these genes are expressed in class III da neurons.

934C A drosophila model depicting braak-like propagation of tau pathology *Aarya Vaikakkara Chithran*[1], Ben Batchelor[2], Benjamin Boehme[1], Lovesha Sivanantharajah[3], Tianshun Lian[1], Brad Richardson[2], Eva Ruiz[2], Efthimios Skoulakis[4], Amrit Mudher[2], Douglas Allan[1] 1) Department of Cellular and Physiological Sciences, University of British Columbia, Vancouver, BC, Canada; 2) Department of Biological Sciences, University of Southampton, Southampton, United Kingdom; 3) Department of Biological Sciences, University of Bangor, Bangor, United Kingdom; 4) Biomedical

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Prion-like propagation through circuits is believed to be the mechanism by which tau pathology spreads throughout the brain in tauopathies like Alzheimer's disease (AD). This is reflected in the neuropathological Braak-staging of disease and manifests in the progressive cognitive decline evident clinically. Though various synaptic proteins are implicated, the precise players and mechanism(s) mediating the trans-cellular spread of pathological tau species remain unclear. Furthermore, though the trans-cellular spread of pathological tau species has been demonstrated in many experimental models, the neurobiological consequences in recipient neurons are largely unknown. Moreover, in all such studies, the tau species that propagates is invariably mutated or isolated from pathological fractions of brains of tauopathy patients. This is puzzling given that it is wild-type tau that becomes pathological and spreads in AD, and this process is accompanied by neurodegeneration. We report a novel Drosophila model in which wildtype human tau expressed in select neuronal subsets becomes pathological and undergoes trans-cellular spread through adult brain circuits, causing neurodegeneration reminiscent of late stages of disease in AD brain. The superior genetic tractability of this model makes it ideally suited for dissection of the key players that mediate this pathogenic process through genetic and pharmacological modifier screens. Furthermore, the availability of functional and behavioural assays for many adult brain circuits will enable future studies to reveal the neurobiological consequences of spreading tau pathology more directly.

935A DDX17 modulates FUS toxicity in an RGG-domain dependent manner Tyler Fortuna[1], Sukhleen Kour[1], Eric Anderson[1], Caroline Ward[1], Dhivyaa Rajasundaram[2], Christopher Donnelly[3], *Udai Pandey*[1] 1) Department of Pediatrics, Children›s Hospital of Pittsburgh of UPMC; 2) Division of Health Informatics, Department of Pediatrics, Children's Hospital of Pittsburgh, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA; 3) Department of Neurobiology, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA

Mutations in Fused in Sarcoma (FUS) have been linked with juvenile and aggressive form of amyotrophic lateral sclerosis (ALS). Cytoplasmic aggregation and defects in DNA repair pathway have been linked with ALS pathogenesis. However, the molecular mechanisms and genetic modifers of DNA damage repair pathway are yet not known. While doing RNA-sequencing analysis of the *Drosophila* brains expressing FUS, we identified significantly altered genes and pathways involved in FUS-mediated neurodegeneration *in vivo*. We found that the DEAD-Box Helicase 17 (DDX17) expression level was significantly downregulated in response to mutant FUS in *Drosophila* and human cell lines. Interestingly, mutant FUS recruits nuclear DDX17 into cytoplasmic stress granules and biochemically interacts with DDX17 through the RGG1 domain of FUS. Importantly, genetic upregulation of DDX17 reduces cytoplasmic mislocalization and sequestration of mutant FUS into cytoplasmic stress granules. We identified DDX17 as a novel regulator of the DNA damage response pathway whose upregulation repairs defective DNA damage repair machinery caused by mutant neuronal FUS ALS. In addition, we show DDX17 is a novel modifier of FUS-mediated neurodegeneration in vivo. Our findings indicate DDX17 is downregulated in response to mutant FUS, and restoration of DDX17 levels suppresses FUS-mediated neuropathogenesis and toxicity in vivo.

936B Metabolic Dysregulation in Frontotemporal Dementia *Jackson Diltz*, Emily Peterson, Em Teixeira, Angela Brillantes, Taylor Barber, Kristen Burke, Simone Alvares, Madeline O'Connor, Arianna Libby, Maximus Morton, Marla Tipping Providence College

Metabolic reprogramming is a common hallmark of many diseases. In recent years the focus on metabolic change in cancerous tissues has increased. However, fewer studies have investigated the metabolic shifts in neurodegenerative diseases. Metabolic reprogramming in neurodegenerative disease has been well documented and glucose uptake is even used as a key diagnostic indicator for some of these diseases. We are utilizing an established Drosophila model of the neurodegenerative disease, Frontotemporal Dementia (FTD), to investigate metabolic changes using a whole brain energy utilization assay. We are also studying the underlying molecular mechanism by using quantitative PCR to measure expression of metabolic enzymes in the brain and whole body. The goal of this project is to determine if metabolism could be a potential target for treatment of FTD.

937C Uncovering the Mechanisms Behind the Neuroprotective Effect of Glycolysis in a *Drosophila* Model of ALS *Nicholas Mortimore*[1], Suvithanandhini Loganathan[1], Hannah Ball[1], Maria Macias[1], MyDuyen Tran[1], Gabrielle Peterson[1], Gabriel Birchak[1,3], Ernesto Manzo[1,4], Daniela Zarnescu[1,2] 1) Department of Molecular and Cellular Biology, University of Arizona, Tucson, AZ; 2) Department of Neuroscience, University of Arizona, Tucson, AZ; 3) University of Pennsylvania, Philadelphia, PA; 4) Vollum Institute, Oregon Health and Science University, Portland, OR

Amyotrophic Lateral Sclerosis (ALS) is a progressive neurodegenerative disease that affects upper and lower motor neurons primarily in individuals aged forty years or greater. ALS causes loss of motor control, paralysis, and eventually respiratory failure and death within 2-5 years of diagnosis; there is no known cure for this disease. TAR DNA-binding protein (TDP-43) is a pathological marker of ALS and aggregation of TDP-43 is observed in 97% of ALS cases. Our lab has generated a *Drosophila* model of ALS based on overexpression of human TDP-43 in a motor-neuron specific manner which recapitulates key disease phenotypes including cytoplasmic accumulation of TDP-43, neuromuscular

junction (NMJ) abnormalities, decreased lifespan, and locomotor defects. Glycolysis upregulation by an increase in Phosphofructokinase-1 (PFK1; PFK in *Drosophila*) has been shown to have a neuroprotective effect in *Drosophila* models of ALS based on TDP-43 proteinopathy. Here we aim to decipher the mechanisms underlying the restorative effect of PFK overexpression (OE) in ALS. One possible mechanism is that PFK assembles in complexes that support energy demands locally, at synapses. Supporting this scenario are our observations that PFK forms puncta within *Drosophila* NMJs in the context of mutant TDP-43 OE. This is consistent with recent evidence from other groups showing that PFK can form clusters at the synapses of *C. elegans* where it associates with other glycolytic enzymes to form Glycolytic bodies (G-bodies) and supports synaptic vesicle cycling under stress. Our preliminary data support this hypothesis as evidenced by FM1-43 dye uptake experiments showing that PFK OE rescues TDP-43 induced synaptic vesicle cycling deficits. Another possible mechanism is that pyruvate, the end product of glycolysis, which was found to be increased in multiple ALS models may act as a scavenger of reactive oxygen species as shown in cancer cells. We are currently interrogating the composition of G-bodies with mass spectrometry analyses of PFK complexes to further test the hypothesis that G-body composition is altered in the context of TDP-43 proteinopathy. These findings may inform novel therapeutic strategies based on improved cellular energetics for ALS and related neurodegenerative disorders.

938A A CRISPR-Cas9 Mediated Knockout of *RNaseZ* in *Drosophila* Neurons *Max Luf*, Ekaterina Migunova, Jake Nelson, Edward Dubrovsky Department of Biological Sciences, Fordham University

The *RNaseZ* gene is a vital and highly conserved gene with homologs in all eukaryotes. It plays an essential role in the maturation of tRNA, being the only enzyme to process the 3'-ends of pre-tRNA in both the nucleus and mitochondria. Point mutations in the human homolog, *ELAC2*, have been linked to a heterogeneous group of neurological conditions, such as microcephaly and untreatable epilepsy. Because these conditions result in such a poor quality of life for patients, we seek to understand what unique role RNaseZ may play in the nervous system.
Here we report the importance of RNaseZ to the *Drosophila* nervous system and demonstrate its role using neuron-specific knockouts of *RNaseZ*. To determine if point mutations of *RNaseZ* homologous to those in humans could elicit neurological dysfunction in Drosophila we first generated two transgenic lines (gZ^{F154L} and gZ^{T520I}) carrying mutant alleles of *RNaseZ* under its natural promoter. When the endogenous RNaseZ is removed these transgenes led to increased neurodegeneration in adult flies, decreased motor ability, and shortened life span. To then determine if this process was cell-autonomous, we generated a Cas9-mediated pan-neuronal knockout (KO) of *RNaseZ* in all *Drosophila* neurons. This resulted in developmental delays, debilitating motor deficits, and death shortly after eclosion. Because of the dual role of RNaseZ in the mitochondria and the nucleus, we created a Cas9-resistant rescue construct and used it to restore wt RNaseZ exclusively to the nucleus, creating a mitochondrial KO of *RNaseZ* in *Drosophila* neurons. The mitochondrial KO of *RNaseZ* produced a phenotype in longevity and locomotive ability almost identical to that of flies with the pan-neuronal KO. Overall, our findings improve our understanding of the unique role that RNaseZ plays in the nervous system and demonstrate how tissue-specific Cas9 engineering and Cas9-resistant rescue constructs can be applied to the modeling of neural degeneration in *Drosophila*.

939B Mechanism of adult neurodegeneration in *drop-dead* mutants *Unmila Jhuti*, Edward Blumenthal Marquette University, Milwaukee, WI

Adult neurodegenerative diseases such as Alzheimer's and Parkinson's are one of the main causes of death for elderly population worldwide. Using *Drosophila* models of neurodegeneration (ND) to study such diseases has been proven useful because they show striking similarities to the human diseases. This project aims to understand ND in flies mutant for *drop-dead* (*drd*). Absence of *drd* expression is associated with adult ND and short lifespan, but the molecular pathways responsible for ND in this model have not been identified. Hyperactivation of innate immunity (HII) has been linked to ND both in human diseases and in *Drosophila*. It is yet to be identified how HII pathways are activated in adult neurodegenerative diseases. Preliminary data suggest a causal connection of HII with ND in *drd* mutants. Expression of the antimicrobial peptide genes *DptB*, *CecA1* and *AttC* was increased by 2-4 fold in *drd* mutant brains compared to heterozygous sibling controls. Furthermore, *drd* mutant flies that are heterozygous for mutations in the immune genes *rel* or *imd* showed an increased median lifespan (8-10 days) compared to *drd* mutant controls (4-7 days). Although ND has been identified in mutant *drd* brains, the spatiotemporal pattern and pathway(s) of cell death have not been characterized. Immunostaining with the apoptotic marker Dcp-1 suggested activation of caspase-dependent apoptosis in the brains of *drd* mutants as early as the day of eclosion (P0). Intense and spatially dispersed staining was observed in older (P2-3) flies. Staining with the non-specific cell death marker acridine orange revealed widespread cell death beginning at P0.
Previous work has shown that expression of *drd* in the respiratory tracheae is necessary and sufficient to prevent ND. Thus, brain cell death could potentially be connected to disruption of gas exchange and hypoxia. However, staining mutant brains with the ROS marker dihydroethidium showed the presence ROS only in older flies (P2-3), but not in younger flies (P0), suggesting that hypoxia is not a primary cause of ND in *drd* mutants.
Studying the role of *drd* will provide significant insight into the link between HII and adult ND. Identification of the pathways leading to cell death can help to understand cellular mechanisms potentially responsible for adult

neurodegeneration.
Supported by Marquette University and the Northwestern Mutual Data Science Institute.
Keywords: innate immunity, apoptosis, cell death, hypoxia, neurodegeneration.

940C The ketone body beta-hydroxybutyrate ameliorates molecular and behavioral pathological markers in a Drosophila model of glial tauopathy. *Celya D. Dahmani*[1], Julia O'Connell[2], Timothy O'Toole[1], Kenneth J. Colodner[2], Geoffrey R. Tanner[1,3] 1) University of Connecticut, Storrs, CT; 2) Mount Holyoke College, South Hadley, MA; 3) Institute for the Brain and Cognitive Sciences, Storrs, CT

Tauopathies are a class of neurodegenerative diseases characterized by abnormal intracellular aggregations of the microtubule-stabilizing protein tau. Symptomology of tauopathies features impaired cognition—including difficulties with learning and memory—caused by progressive neuronal and synaptic degeneration. Tau pathology is often characterized by an accumulation of hyperphosphorylated tau protein resulting in the formation of neurotoxic tau aggregates, also known as neurofibrillary (NFT) and gliofibrillary (GFT) tangles. These aggregates may hinder neural glycolytic metabolism and result in degradation of the myelin sheath and other glial components. Ketone bodies (KBs), metabolites of the putatively-neuroprotective high-fat and low-carbohydrate ketogenic diet (KD), bypass cytoplasmic glycolysis and enter directly into mitochondrial metabolism. The KD and KBs serve to elevate cellular ATP/ADP ratio and thus may rescue neural energetics. We have obtained evidence strongly suggesting that KBs may exhibit neuroprotective effects in a Drosophila melanogaster model of traumatic brain injury (TBI); repeated TBI may lead to the progressive neurodegenerative condition termed Chronic Traumatic Encephalopathy (CTE), which is characterized in part by intracellular aggregation of tau protein in neural cells. We employed a Drosophila model of tauopathy that allows for spatial restriction of exogenous tau protein overexpression to glial cells and for temporal restriction of tau overexpression to adulthood, so as to avoid developmental effects of neural tau protein overexpression. We directly applied a racemic mixture of the KD metabolite beta-hydroxybutyrate (β-HB)—the major circulating ketone body (KB)—to a standard high-carbohydrate Drosophila diet to determine whether dietary KB supplementation can ameliorate tauopathy-associated learning deficits. We found that β-HB markedly improves tauopathy-induced learning deficits in mixed-sex groups of adult Drosophila and reduces levels of phosphorylated tau in the brains of male flies subjected to induced glial tauopathy.

941A Phagocytic glia mediate prion-like spreading of mutant huntingtin aggregates in *Drosophila* brains Kirby Donnelly, Aprem Zaya, Graham Davis, Olivia DeLorenzo, *Margaret Panning Pearce* University of the Sciences

A key pathological and diagnostic feature of most neurodegenerative diseases is appearance of insoluble aggregates due to protein misfolding in the central nervous system (CNS). Accumulation of toxic protein aggregates in the brain is partially offset by a number of clearance mechanisms, including the ubiquitin-proteasome pathway, autophagy, and engulfment by phagocytic glia. A growing body of evidence supports the hypothesis that pathogenic aggregates associated with many neurodegenerative diseases behave similarly to infectious prions--they spread from cell-to-cell and nucleate the aggregation of natively-folded versions of the same protein, events which are thought to contribute to propagation of aggregates in the brain. We have recently reported that mutant huntingtin (mHTT) aggregates associated with the inherited neurodegenerative disorder Huntington's disease spread in a "prion-like" manner between synaptically-connected neurons and glia in the *Drosophila* CNS. mHTT aggregates formed in presynaptic olfactory receptor neuron (ORN) axons directly nucleate the aggregation of soluble wild-type HTT (wtHTT) proteins expressed in post-synaptic partner projection neurons (PNs) or nearby glia. ORN-to-PN and ORN-to-glia transfer of mHTT aggregates is enhanced when ORN activity is silenced, slowed when caspase-dependent apoptosis in ORNs is blocked, and remarkably, require expression of the conserved glial scavenger receptor Draper/MEGF10. Further, mHTT aggregate transmission between synaptically-connected ORNs and PNs requires a transient visit to the glial cytoplasm, indicating that phagocytic glia directly mediate aggregate transfer between neurons in vivo. Preliminary data from our lab indicate that entry of phagocytosed neuronal mHTT aggregates into the glial cytoplasm involves certain Rab GTPases with proposed roles in intracellular vesicle membrane fusion related to phagosome maturation, suggesting that inefficient or incomplete progression of engulfed material in the phagolysosomal system may generate seeding-competent mHTT aggregate species in the glial cytoplasm. In support of this, expression of mHTT in ORNs accelerates age-related decline in glial phagocytic clearance of axonal debris. Together, our findings add to a growing understanding of phagocytic glia as double-edged players in neurodegeneration—these cells clear neurotoxic aggregates, but age- and/or disease-related defects in this process can paradoxically promote aggregate propagation in the brain.

942B Dynamic transcriptional changes in the adult *Drosophila* central nervous system highlights potential coordination of stress and repair responses following traumatic brain injury *Eddie Cho*[1], Alec Candib[1], Reina Hastings[1], Gina Torabzadeh[1], Jesse Rojas[1], Sharon Zhang[1], Marta Lipinski[2], Eric Ratliff[1], Kim Finley[1] 1) Shiley BioScience Center and Biology Dept, San Diego State University, San Diego, CA ; 2) Department of Anesthesiology, University of Maryland School of Medicine, Baltimore, MD

Traumatic brain injury (**TBI**) from accidents, domestic violence, sports and combat are a major cause of worldwide

mortality and disability. Several groups have shown that adult *Drosophila* can be an effective model to examine the physiological, behavioral, neuronal, and molecular responses following TBI exposure. In this study, we have continued our Drosophila TBI research using RNA-sequencing to examine dynamic acute and long-term changes to central nervous system (**CNS**) gene expression profiles in adult flies following severe (**sTBI**) and mild repetitive TBI (**mTBI**) exposure. Principal component analysis (**PCA**) highlight highly dynamic transcriptional changes in male and female flies, with over a third of the *Drosophila* transcriptome showing 1.5-fold (+/-) alterations 4-hrs post-TBI. By 24-hrs, both genders rapidly shifted toward baseline transcriptome levels. 4-days post TBI male fly cohorts maintained a more baseline transcriptome profiles, while female flies had a second round of gene expression changes. Transcriptional changes trend toward baseline levels at 7-days post. Genes with significant alterations were analyzed using DAVID to identify functional gene-pathway clusters. At 4-hrs both genders had significant changes to NFKB and IMD pathway components, indicating rapid activation of stress and inflammatory signaling. At this time dramatic changes to neuronal/ERAD stress responses, ribosomal, vesical trafficking, and membrane transport components also occurred. At 24-hrs, inflammation remains elevated, along with alterations to cytoskeletal, kinase-phosphatase and proteolytic pathway members. At 4-days, there is a significant increase in select metallopeptidases as well as changes to organelle, protein and basement membrane modifying processes. 7-days post-TBI, DAVID revealed changes to alternative splicing factors, ER, cytochrome P450, glutathione metabolism, and members of the scavenger receptor pathways. Cell cycle components demonstrated multiplex differences, with PCNA and several cyclin genes increased significantly (4-hrs). Our novel trauma injury paradigm illustrates the effectiveness of model systems to identify conserved genetic factors influencing the complex stress and repair mechanisms underscoring *in vivo* trauma exposure. The goal of these findings is to identify genetic factors that are linked to adverse acute or protracted TBI outcomes and in the discovery of novel therapies for those experiencing trauma.

943C Assessing Novel Therapeutics with a *Drosophila* Model of Neural Aging and Stressors *Alec Candib*, Brandon Molina, Jessica Mastroianni, Nicholas Lee, Natasha Sam, Eddie Cho, Kyle DeAlva, Andrea Gonzalez, Gina Torabzadeh, Josephine Vu, Joy Phillips, Kim Finley Department of Biology, San Diego State University, San Diego, CA

A wide range of potential therapeutics, including modified diets, cannabis, turmeric, and probiotics, are being examined as treatments for disorders associated with aging and neural stress. In this report, we detail a unified *in vivo* method to test the safety, efficacy, therapeutic dosage, and potential molecular mechanism of neuroprotective products using adult *Drosophila melanogaster* as a model system. This includes an integrated three-part platform that examines the impact of individual compounds and biologics on neural aging, TBI responses, and cytotoxic β-amyloid (Aβ). Adult flies were administered different dosages of cannabis-derived cannabinoids (CBD, CBN, THC), select drugs (J147, metformin, donepezil), and active and inactive biologics (LGG, IAB). Aging studies show select dosages of several therapies preserved locomotor behaviors (negative geotaxis response, NGR) and promoted longevity, while other therapies exacerbated age-related NGR decline and reduced longevity. Pre-treatment with several therapies also helped preserve NGR profiles and promote longevity in traumatized fly cohorts. To examine the potential molecular mechanisms of these compounds, changes to the autophagy and inflammation (NF-κB) pathways were investigated in adult tissues. Several compounds impaired basal and fasting-induced autophagic flux, while others increased autophagic flux and reduced protein aggregate formation. Several therapies reduced the expression of downstream NF-κB signaling targets (antimicrobial peptides) after TBI. The impact of CBD, IAB, and Donepezil on longevity, NGR, NF-κB signaling, and autophagy were studied in flies expressing Aβ peptides. Together, these studies showed that these compounds have a significant impact on *Drosophila* physiology and molecular pathways. Generally, exposure to every compound but THC had similar neuroprotective and longevity-promoting effects, while THC had a limited positive impact only following mTBI. These findings demonstrate the versatility of *Drosophila* as a drug discovery model system and highlight the need for future studies to clarify the molecular interactions and downstream consequences of natural products on complex biological systems and tissues.

944A Identify novel approaches suppressing stress granule assembly to mitigate TDP-43-mediated neurotoxicity *Quinlan Mewborne*, Jessica Chalk , Junli Gao, Ke Zhang Mayo Clinic Jacksonville

Amyotrophic lateral sclerosis (ALS) is a degenerative disease of the motor neurons sometimes associated with frontotemporal dementia (FTD). These two neurodegenerative diseases are progressive, increasingly prevalent, and currently incurable. A major challenge of the therapeutic development of these diseases is our incomplete understanding of the pathogenic mechanism. Previous studies have suggested that stress granules (SGs), RNA/protein condensates assembled in cells under stress, play a critical role in ALS/FTD pathogenesis. Consistent with this notion, chemical inhibitors suppressing SG assembly suppress neurodegeneration in cellular and animal models of ALS/FTD. However, these inhibitors exhibit detrimental side effects in mammals in preclinical studies, preventing their translation into clinics. Thus, we propose to use a fly model to identify novel ways targeting SG assembly as potential therapeutic strategies for ALS/FTD.

Tar DNA-binding protein 43 (TDP-43) is an SG protein, whose cytoplasmic aggregation has been implicated in ~98% of

ALS and ~45% of FTD. Indeed, SG assembly has been shown to trigger TDP-43 aggregation in multiple models of ALS/FTD. Consistent with these data, overexpressing a cytoplasmic form of TDP-43 causes eye degeneration in flies. Using this model, we screened through the RNAi of 250 fly genes whose human homolog have been shown to promote SG assembly in cultured cells. We identified ~20 genes whose RNAi strongly or moderately suppresses TDP-43-mediated eye degeneration in our flies and will further characterize their roles in ALS/FTD pathogenesis.

945B Poly(ADP-ribose) Promotes the Condensation of *C9ORF72* Arginine-rich Dipeptide Repeat Proteins *Ke Zhang*, Junli Gao, Ritika Punathil, Tania Gendron, Udit Sheth Mayo Clinic Florida

Arginine-rich dipeptide repeat proteins (R-DPRs), abnormal translational products of a GGGGCC hexanucleotide repeat expansion in *C9ORF72*, play a critical role in *C9ORF72*-related amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD), the most common familial form of ALS and FTD (c9ALS/FTD). R-DPRs undergo phase separation to form condensates *in vitro* and, as a c9ALS/FTD pathological hallmark, aggregate and sometimes co-aggregate with TDP-43 in patients. However, how these processes are regulated is unclear. Here, we show that poly(ADP-ribose) (PAR) interacts with R-DPRs, promotes R-DPR phase separation and toxicity *in vitro*, and correlates with insoluble R-DPR and TDP-43 in patient postmortem tissue. Loss of PAR suppresses R-DPR aggregation and neurodegeneration in fly or cellular models of c9ALS/FTD. Our findings identified PAR as a pathogenic contributor promoting R-DPR condensation both *in vitro* and *in vivo*, suggesting the potential of R-DPR condensation as a target for c9ALS/FTD therapeutic intervention.

946C Behavioral changes and tau pathology in response to traumatic brain injury in Drosophila *Roilea Maxson*[1], Christine Smoyer[1,2], Kailea Wiese[1], Kassandra Ori-McKenney[1] 1) University of California, Davis; 2) University of Kansas

Traumatic brain injury (TBI) is a disruption of normal brain function that results from mild to severe impacts to the head, and can affect memory, as well as behaviors such as anxiety, aggression, and depression. In addition, TBI is the leading risk factor for late onset neurodegenerative diseases such as Alzheimer's Disease. One major hallmark of TBI is the presence of hyperphosphorylated tau in the brain and cerebrospinal fluid. In a healthy brain, tau is a microtubule associated protein that controls microtubule based processes, but upon hyperphosphorylation, tau forms aggregates called neurofibrillary tangles (NFTs) that are thought to contribute to neurodegeneration. However, the series of events that cause tau hyperphosphorylation and NFT formation in response to injury, and how these contribute to neurodegeneration are not well established. We are utilizing a high impact method to subject flies expressing 2N4R human tau pan-neuronally to TBI in an effort to study the contribution of tau phosphorylation and oligomerization to neurodegeneration and behavioral decline. At 24 hours after inflicting TBI on 1 week old virgin males, we recorded flies to observe courtship and aggression. We found that pan-neuronal expression of tau caused an increase in inter-male aggression in flies subjected to TBI, both in terms of the amount of time engaged in aggressive acts and in the total number of aggressive acts. We have screened a variety of specific drivers for neuronal types known to be involved in mating and aggressive behaviors, and have identified dopaminergic and serotonergic circuits as contributors to increased aggression. In addition to our behavioral studies, we are performing immunohistochemistry on fly brains from 24 hours, 1 week, or 3 weeks post-injury to determine if there are any differences in tau localization and/or neurodegeneration. The neurodegeneration associated with TBI and tau expression in Drosophila can be indicated via the presence of vacuoles. We aim to determine if coupling TBI and tau expression affects tau localization or exacerbates the occurrence of neurodegeneration seen with TBI or tau expression alone. In addition, we are analyzing the phosphorylation and aggregation of tau isolated from fly brains using biochemical techniques. Ultimately, we hope to elucidate how different molecular states of tau contribute to neurodegeneration and lead to alterations in brain function and behaviors.

947A Comparing the Neurotoxic Effects of P3 ($A\beta_{17-42}$) and $A\beta_{1-42}$ using *Drosophila* as an Alzheimer's Disease Model *Marisa Fujimoto*, Alfredo Rojas Moreno, Yuhao Pan, Emmanuel Akabogu, Gloria Qui, Joey Wong, Katrina Haas, Selma Alamarie, Tatyanah Farsh, Annika Streeb, Jeremy Lee Department of Molecular, Cell, and Developmental Biology, University of California, Santa Cruz, CA

Alzheimer's disease (AD) is a neurodegenerative disease associated with nearly 6 million deaths in the U.S. every year. AD is characterized by a loss of memory, decline in cognitive abilities, and changes in mood and personality. A hallmark of AD pathology is the presence of Aβ plaques, which are formed by the aggregation of Aβ peptides. The $A\beta_{1-42}$ peptide is produced by the cleavage of amyloid precursor protein (APP), first by β-secretase and then γ-secretase. An alternative pathway uses α-secretase instead of β-secretase and generates a peptide called P3 ($A\beta_{17-42}$), that is 16 amino acids shorter than $A\beta_{1-42}$ and is composed of the hydrophobic portion of $A\beta_{1-42}$. This P3 cleavage pathway is often characterized as non-amyloidogenic, however, recent evidence suggests P3 is amyloidogenic (Kuhn, et al., 2020). As such, there is reason to think that P3 could have similar deleterious effects as $A\beta_{1-42}$. The purpose of our research is to characterize P3 peptide's neurotoxicity and determine whether it interacts with Aβ. To investigate this, we generated P3 expressing flies, $A\beta_{1-42}$ expressing flies, and $A\beta_{1-42}$/P3 co-expressing flies, in which the peptides were expressed pan-neuronally, to examine the neurodegenerative effects of these two peptides independently, as well as their synergistic neurotoxic effects. RT-PCR experiments have confirmed expression of the transgenes. A longevity assay was conducted to measure lifespan in the transgenic flies and the Rapid Iterative Negative Geotaxis (RING) assay was performed to

monitor their locomotor performance in relation to age. These results, along with electron micrograph analysis of the peptides' effects when expressed in fly eyes, indicate that P3 expression alone has similar, but less severe, effects as Aβ on lifespan, behavior, and neurodegeneration. Our results also suggest that P3 exacerbates the effects of $A\beta_{1-42}$ when co-expressed. RNA sequencing is being used to further investigate the effects of these peptides by examining whether the expression of neurodegenerative-related genes and neuroplasticity-related genes, known to be affected by $A\beta_{1-42}$, are similarly affected by P3. We will also conduct co-immunoprecipitation experiments using $A\beta_{1-42}$/P3 co-expressing flies to determine whether the two peptides directly interact. Our research will help to determine whether P3 might be a direct contributing factor to AD pathology and/or have an effect via its interaction with Aβ.

948B Observing the Effects of the Human Peptide, LL-37, on $A\beta_{42}$'s Neurotoxicity and Effects on Gene Expression Using a *Drosophila* Model of Alzheimer's Disease *Ruby Guevara*, Kenneth Owyang, Jennifer Tan, Marissa Joe, Taylor Jones, Duyen Nguyen, Emmanuella Tetteh, Shannon Twardy, Celine Neudorf, Shelly Huynh, Jeremy Lee Department of Molecular, Cell, and Developmental Biology, University of California, Santa Cruz, CA

The hallmark of Alzheimer's disease (AD) is the presence of amyloid plaques in affected areas of the brain, which are aggregates of the amyloid-beta (Aβ) peptide. Aβ, especially $A\beta_{42}$, is known to be neurotoxic and crucial in AD pathology. While the function of Aβ is not well understood, recent studies have shown it to have antimicrobial effects (Kumar et al., 2016). It has also been shown to interact with the human antimicrobial peptide LL-37 in binding assays in vitro (De Lorenzi, et al., 2017). If LL-37 and Aβ interact in vivo, it may affect the neurotoxicity of Aβ.
In order to determine whether LL-37 affects Aβ neurotoxicity, we generated four transgenic flies pan-neuronally expressing human $A\beta_{42}$ and/or LL-37: LL37-expressing, $A\beta_{42}$-expressing, $A\beta_{42}$/LL-37 co-expressing, and non-expressing controls. Longevity assays and RING climbing assays were conducted to determine whether LL-37 has an effect on $A\beta_{42}$'s neurotoxicity in vivo. The results showed that co-expression of LL-37 and $A\beta_{42}$ led to longer lifespans than expression of only $A\beta_{42}$, but shorter lifespans than non-expressing controls. In developmental assays, these co-expressing flies had higher survivorship to eclosion than $A\beta_{42}$-expressing flies. In addition, RING assays showed that dual-expressing flies had a significant improvement in locomotive functions compared to LL37-expressing and $A\beta_{42}$-expressing flies. These results indicate that LL-37 might attenuate the deleterious effects of $A\beta_{42}$ in Alzheimer's disease.
We are assessing LL-37 and $A\beta_{42}$ expression levels in our transgenic flies by qRT-PCR and Western blot to determine whether co-expression of LL-37 has an effect on $A\beta_{42}$ transcript or protein levels. We also intend to assess whether the expression of LL-37 modulates Aβ's known effects on the expression of a variety of genes involved in synaptic plasticity and neuronal function. To do this, we are performing RNA sequencing analysis to obtain a quantitative snapshot of RNA transcripts present within our four genotypes. Using a baseline comparison of $A\beta_{42}$-expressing flies versus wild type, we will focus on key genes of interest previously shown to be affected by $A\beta_{42}$. This will allow us to compare any modulating effect LL-37 has on $A\beta_{42}$'s effects on gene expression, by assessing co-expressing flies and noting any key differences in their RNA expression profiles. This will help provide insight into the mechanism by which LL-37 attenuates Aβ's neurotoxic effects.

949C An Analysis of the Microbiota of Various *Drosophila melanogaster* Parkinson›s Disease Models *Evan Marshman*[1], Samara Petersen[1], Zachary Pickup[3], Gerald Call[2], John Chaston[1] 1) Department of Plant and Wildlife Sciences, College of Life Sciences, Brigham Young University, Provo, UT; 2) Department of Pharmacology, College of Graduate Studies, Midwestern University, Glendale, AZ; 3) Biomedical Sciences Program, College of Graduate Studies, Midwestern University, Glendale, AZ

The gut-brain axis is defined as the bi-directional interactions that occur between the brain and the gut of an organism. Considering this interaction, the brain is also known to affect the gut microbiome of an organism. The microbiome has specifically been shown to be affected by many neurological diseases. Parkinson's disease (PD) is a neurodegenerative disease that affects motor function. We are interested in studying the interactions between the gut microbiome and PD. Current research has shown that the microbiome of an individual with PD differs from the microbiome of a healthy individual. This has been exhibited in humans and mice. We predicted that we would detect this same variation in the microbiota of *D. melanogaster* (fruit fly) models of PD, relative to a genetically matched control. To test this hypothesis, we used five PD fruit fly models obtained from four different laboratories, each mimicking human PD by different mutations in their genome. We collected and analyzed 16S rRNA sequence, reporting the microbiota composition in our fly samples. We found various microbes that were differentially abundant between control and PD models at a genus level. We also show that the bacterial diversity of PD models is overall significantly lower than in control flies, consistent with the idea that individuals with declined health show lower diversity in their gut microbiome. The major conclusion of our work is that there is significant variation in microbiota composition between conventionally reared fly models of PD and their controls. This shows that the differences seen in humans and other models are also present in fruit flies.

950A A photo-switchable assay system for dendrite degeneration in *Drosophila melanogaster* *Han-Hsuan Liu*[1], Chien-Hsiang Hsu[2], Lily Jan[1], Yuh-Nung Jan[1] 1) Howard Hughes Medical Institute, Departments of Physiology, Biochemistry and Biophysics, University of California, San Francisco, San Francisco, CA, United States.; 2) Department of Pharmaceutical Chemistry, University of California, San Francisco, San Francisco, CA, United States.

Neurodegeneration arising from aging, injury or disease has devastating health consequences. Whereas neuronal survival and axon degeneration have been studied extensively, much less is known about how neurodegeneration impacts dendrites. To develop an assay for dendrite degeneration in the *Drosophila* peripheral nervous system, we used photo-switchable caspase-3 (caspase-LOV) to induce neurodegeneration with tunable severity by adjusting illumination duration in class 4 dendrite arborization (c4da) neurons during development and adulthood. We found that c4da neurons were able to sustain mild caspase-3 induced neurodegeneration and continue to grow. To ask whether mechanisms underlying axon degeneration also govern dendrite degeneration, we tested the involvement of the Wallerian degeneration pathway by examining the effects of expressing the mouse Wallerian degeneration slow (Wld^S) protein in larval and adult c4da neurons. Here we report Wld^S expression protect c4da neurons from caspase-3 induced dendrite degeneration in larval and adult c4da neurons through reducing the dendrite elimination without affecting dendrite addition. At the functional lever, Wld^S expression improved the recovery of thermal nocifensive behavior that was impaired by chronic low-level of caspase-LOV activity. By establishing ways to induce graded dendrite degeneration, we uncover a protective role of Wld^S in caspase-3 induced dendrite degeneration.

951B TDP-43 expression in dementia-relevant circuits causes axonal degeneration and behavioral deficits in Drosophila *Reed Bjork*[1], Rebekah Keating Godfrey[1], Christi Williams[1], Hillary Cowell[1], Allison Michael[1], Grace Halaufia[1], Erik Lehmkuhl[1], Eric Alsop[2], Kendall van Keuren-Jensen[2], Daniela Zarnescu[1] 1) The University of Arizona; 2) The Translational Genomics Research Institute

Trans active response (TAR) DNA-Binding protein 43 (TDP-43), an evolutionarily conserved RNA/DNA binding protein that regulates RNA processing, is known to form pathological, cytoplasmic inclusions in numerous neurodegenerative diseases. These cytoplasmic accumulations, collectively referred to as TDP-43 proteinopathy, are a hallmark of motor neuron degeneration in amyotrophic lateral sclerosis (ALS, 97% of cases) and are also detected in nearly half of frontotemporal dementia (FTD) and Alzheimer's disease (AD) cases, establishing TDP-43 as an important candidate for modeling neurodegenerative disease. Drosophila models of TDP-43 proteinopathy in ALS and other neurological disorders have elucidated important molecular dynamics of neurodegeneration, yet such models have not yet been employed to understand TDP-43 dependent pathomechanisms in dementia. Given the striking overlap between ALS and FTD and our own success in modeling TDP-43 proteinopathy in a fly model of ALS, we have developed a novel fruit fly model of dementia by overexpressing human TDP-43 in the mushroom bodies (MBs) of the Drosophila brain. Insect MBs form an elaborate, associative network that overlaps in function and gene expression with neurons in the human hippocampus and frontal cortex, making it an appropriate brain structure to model human dementia. We demonstrate that TDP-43 mislocalizes from the nucleus to the cytoplasm and forms axonal inclusions accompanied by age-dependent behavioral deficits and axonal degeneration in MBs. Importantly, cognitive deficits in working memory and sleep appear prior to our ability to detect axonal degeneration, paralleling what is seen in human disease. Based on recent studies identifying a role for TDP-43 in translation dysregulation, we sought to identify the molecular targets of TDP-43 in the MBs using RNA immunoprecipitations and RNAseq. These experiments identified several candidate targets of TDP-43 in mushroom bodies, including Dally-like protein (Dlp/GPC6), a translational target that we recently reported in Drosophila ALS models and patients. These findings suggest that TDP-43 overexpression in MBs causes FTD-relevant phenotypes that may help uncover novel mechanisms of disease. Future experiments are aimed at identifying additional FTD-relevant targets of TDP-43, including novel therapeutic strategies.

952C A small molecule ion channel screen to suppress gliopathic epilepsies *Walt Krueger*[1,2], Bidisha Roy[1], Jungsoo Han[3], Benjamin Geier[1,2], Lawrence Reiter[1,4,5] 1) Department of Neurology, UTHSC, Memphis, TN; 2) IBSP Program, UTHSC, Memphis, TN; 3) Department of Biochemistry, UT Southwestern, Dallas, TX; 4) Department of Pediatrics, UTHSC, Memphis, TN; 5) Department of Anatomy and Neurobiology, UTHSC, Memphis, TN

Duplication 15q syndrome (Dup15q) is caused by the presence of at least one extra copy of the 15q11.2-q13.1 region. Characteristics of Dup15q include hypotonia, intellectual disability, autism spectrum disorder and, in the majority of isodicentric 15 cases, pharmacoresistant epilepsy. We previously constructed a fly model that successfully recapitulates the seizure phenotype observed in Dup15q individuals by overexpressing Drosophila Ube3a (Dube3a) in glial cells. To identify new anti-epileptic drugs, our lab developed a medium throughput screening method to repurpose previously FDA or otherwise approved chemical libraries for their ability to suppress seizures. We recently used this model to screen 1,280 compounds from the Prestwick Chemical Library. Eight compounds were identified that reduce seizure recovery time by at least 50% in both male and female flies. Most of these compounds act through serotonin or dopamine receptors to increase Na^+/K^+-ATPase activity in glial cells (Roy *et al.* (2020) *Biol Psychiatry* 88(9):698-709). In the current study, we evaluated 70 compounds from the Screen-Well Ion Channel Ligand Library for their ability to suppress seizures in our glial cell specific epilepsy model. The primary screen was composed of 24 calcium channel modulators, 23 potassium channel modulators, 10 sodium channel modulators, 7 intracellular calcium modulators, and 6 other miscellaneous drugs. We identified 8 compounds that suppress seizures in *repo>Dube3a* flies by at least 50%. Seventy-five percent of these compounds are potassium modulators and 25% are calcium modulators. ATP-sensitive Inward Rectifying K+ channel (KATP) modulation is a shared commonality among 3 of the 8 compounds. To evaluate the

potentially critical role KATP modulation plays in seizure suppression, as well as to investigate the importance of glial-specific neuronal modulation, we are currently testing drug efficacy on flies that simultaneously express Dube3a and an RNAi against the *Drosophila* homologue for KATP (*Irk*) in glial cells. We expect to find that KATP modulators fail to suppress seizures in the absence of sufficient irk channel expressions. These studies will lead directly to new candidate drugs, specific ion channel agonists and antagonists, that may eventually be used clinically to suppress seizures in Dup15q syndrome.

953A Characterization of the Fly Models for *Glutaminase*-related Neurological Disorders *Zelha Nil*[1, 2], Carlos Ferreira[3], Camilo Toro[4], David Adams[5], Oguz Kanca[1, 2], Shinya Yamamoto[1, 2], Hugo Bellen[1, 2], Undiagnosed Diseases Network 1) Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX, USA; 2) Jan and Dan Duncan Neurological Research Institute, Texas Children's Hospital, Houston, TX, USA ; 3) Metabolic Medicine Branch, National Human Genome Research Institute, National Institutes of Health, Bethesda, MD, USA; 4) Adult NIH Undiagnosed Diseases Program, National Human Genome Research Institute, National Institutes of Health, Bethesda, MD, USA; 5) Human Biochemical Genetics Section, National Human Genome Research Institute, National Institutes of Health, Bethesda, MD, USA

Glutaminase (GLS) encodes a mitochondrial enzyme that converts L-glutamine into L-glutamate in mitochondria. In addition to serving as building blocks of proteins, these amino acids have many additional roles *in vivo* including maintaining the cellular redox status, fueling the Krebs cycle, and synaptic transmission. Recent studies have identified rare human diseases that are caused by loss- (LOF) or gain-of-function (GOF) variants in *GLS*. Due to the pleiotropic roles of glutamine, glutamate and other related metabolites in diverse cellular processes, the precise molecular mechanisms underlying the human diseases are unknown. Here, we generated and characterized different alleles of fly *GLS* and transgenic human *GLS* expressing lines to study these disorders. We find that complete loss of *GLS* (G*LS*$^{Gal4\Delta All}$) or the loss of the isoforms with strong mitochondrial targeting signals (G*LS*$^{\Delta Mito}$) do not cause any obvious defects. However, a dominant negative allele expressing the short N-terminal fragments of different *GLS* isoforms (G*LS*^{DN}) shows decreased survival, shortened life-span, reduced body size, progressive loss of motor skills and glutamine accumulation similar to diseases caused by some *GLS* missense variants in humans. Moreover, we found that the ubiquitous overexpression of a potential GOF variant (p.Ser482Cys) in human *GLS* in wild-type flies is toxic, whereas overexpression of the reference or predicted LOF variants do not show any effect. These preliminary data argue that *Drosophila* is a promising model to explore molecular mechanisms of rare human *GLS* diseases.

954B Biallelic variants in *OGDHL* cause a neurodevelopmental spectrum disease featuring epilepsy, hearing loss, visual impairment, and ataxia Zheng Yie Yap[1], Stephanie Efthymiou[2], Simone Seiffert[3], Karen Vargas Parra [1], Sukyeong Lee[4], Alessia Nasca[5], Reza Maroofian[2], Isabelle Schrauwen[6], Manuela Pendziwiat[7], Sunhee Jung[8], Pasquale Striano[9], Kshitij Mankad[10], Barbara Vona[11], Sanmati Cuddapah[12], Srinitya Gannavarapu[13], Costanza Lamperti[5], Andrea Legati[5], Vincenzo Salpietro[9], Sarah von Spiczak [7], Abigail Sandoval[1], Massimo Zeviani[14], Adi Reich[15], Cholsoon Jang[8], Ingo Helbig[7,16,17], Tahsin Stefan Barakat[18], Daniele Ghezzi[5,19], Suzanne M Leal[6], Yvonne Weber[3,20], Henry Houlden[2], *Wan Hee Yoon* [1] 1) Oklahoma Medical Research Foundation, OKC, OK, USA; 2) UCL Queen Square Institute of Neurology, London, UK; 3) University of Tübingen, Tübingen, Germany ; 4) Baylor College of Medicine, Houston, TX, USA; 5) Fondazione IRCCS Istituto Neurologico "Carlo Besta", Milan, Italy; 6) Columbia University Medical Center, New York, NY, USA; 7) Christian-Albrechts-University of Kiel, Kiel, Germany; 8) University of California Irvine, Irvine, CA, USA; 9) University of Genoa, Genoa, Italy; 10) Great Ormond Street Hospital for Children, London, UK; 11) Eberhard Karls University, Tübingen, Germany; 12) Children's Hospital of Philadelphia, Philadelphia, PA, USA; 13) Western University, London, ON, Canada; 14) University of Padova, Padova, Italy; 15) GeneDx, Gaithersburg, MD, USA; 16) Children's Hospital of Philadelphia; 17) University of Pennsylvania, Perelman School of Medicine, Philadelphia, PA, USA; 18) Erasmus University Medical Center, Rotterdam, Netherlands; 19) University of Milan, Milan, Italy ; 20) University of Aachen, Aachen, Germany

The 2-oxoglutarate dehydrogenase-like protein (OGDHL) is a rate-limiting enzyme in the Krebs cycle that plays a pivotal role in mitochondrial metabolism. *OGDHL* expression is restricted mainly to the brain in humans. Here, we report nine individuals from eight unrelated families carrying biallelic variants in *OGDHL* with a range of neurological and neurodevelopmental phenotypes including epilepsy, hearing loss, visual impairment, gait ataxia, microcephaly and hypoplastic corpus callosum. The variants include three homozygous missense variants (p.Pro852Ala, p.Arg244Trp, p.Arg299Gly), three compound heterozygous single nucleotide variants (p.Arg673Gln/p.Val488Val, p.Phe734Ser/p.Ala327Val, p.Trp220Cys/p.Asp491Val), one homozygous frameshift variant (p.Cys553Leufs*16), and one homozygous stopgain variant (p.Arg440Ter). To support the pathogenicity of the variants, we developed a novel CRISPR/Cas9-mediated tissue-specific knockout with cDNA rescue system for *dOgdh,* the *Drosophila* ortholog of human *OGDHL*. Pan-neuronal knockout of *dOgdh* led to developmental lethality, which was fully rescued by expression of wild-type *dOgdh*. Studies using the *Drosophila* system indicate that Arg673Gln, p.Phe734Ser, and p.Arg299Gly are severe loss-of-function alleles, leading to developmental lethality, whereas p.Pro852Ala, p.Ala327Val, p.Trp220Cys, p.Asp491Val, and p.Arg244Trp are hypomorphic alleles, causing behavioral defects. Transcript analysis from fibroblasts obtained from individual carrying the synonymous variant (c.1464T>C:[p.Val488Val]) in family 2 showed that the synonymous variant

affects splicing of exon 11 in *OGDHL*. Human neuronal cells with *OGDHL* knockout exhibited defects in mitochondrial respiration, indicating the essential role of *OGDHL* in mitochondrial metabolism in humans. Together, our data establish that the biallelic variants in *OGDHL* are pathogenic, leading to a Mendelian neurodevelopmental disease in humans.

955C Proteomic characterization of Dube3a substrates in glia versus neurons using ubiquitin activated interaction trap (UBAIT) *Benjamin Geier*[1], David Kakhniashvili[1], Daniel Johnson[1], Jon Huibregtse[2], Lawrence Reiter[1] 1) University of Tennessee Health Science Center; 2) University of Texas at Austin

Proteolytic activity via the ubiquitin-proteasome system (UPS) serves a crucial role in regulating proteins for degradation and redistribution in the cell. Several human disorders directly result from dysregulation of the UPS. Mutations that affect expression levels of the ubiquitin protein ligase E3A (UBE3A/E6AP) can result in Duplication 15q or Angelman syndrome. While many researchers have paired cell culture methods with immunoprecipitation and blotting techniques to identify UBE3A interactors, to date, only a handful of validated UBE3A substrates have been identified. Through the implementation of a new biochemical method known as a ubiquitin activated interaction trap (UBAIT), we can now covalently link ubiquitin substrates to UBE3A in vivo. We generated two upstream activator sequence (UAS) lines that incorporate a 6x polyhistidine-tagged Dube3a UBAIT construct for expression experiments in all cells (*actin*-GAL4), glial cells (*repo*-GAL4), and neurons (*elav*-GAL4). The first UBAIT is a K48R wild-type ubiquitin line that actively binds ubiquitin substrates and covalently attaches them to His-tagged Dube3a for isolation. The second UBAIT employs a non-functional ubiquitin with a DGG mutation that cannot covalently attach substrates to His-tagged Dube3a, but can serve as a control for other proteins stuck to the expressed UBAITs. Using Ni-NTA column purification combined with unbiased whole proteome analysis we identified 27 proteins using *actin*-GAL4 and 59 proteins using *repo*-GAL4 that appear to be bonafide Dube3a substrates and were not present in the DGG control UBAIT group. These hits included the Na/K pump ATPalpha, which our group previously showed is regulated by Dube3a in glial cells. Other hits include trehalose transporter 1-1(Tret1-1), wings up A(wupA), and rolled(rl). This work marks the first successful implementation of the UBAIT system in a model organism, opening room for a multitude of successive in vivo UBE3A proteomic experiments.

956A Sex and reproductive differences in intestinal tumours *Emily Strachan*[1,2], Irene Miguel-Aliaga[1,2], Susumu Hirabayashi[1,2] 1) MRC London Institute of Medical Sciences, UK; 2) Institute of Clinical Sciences, Faculty of Medicine, Imperial College London, London, UK

Cancer risk, type and progression varies with sex, due to a range of genetic, hormonal and behavioural factors. Post pregnancy this is modulated further due to changes in hormones, growth factors and the immune system.

The *Drosophila* midgut, analogous to the human small intestine, is a highly sexually dimorphic organ. As well as changes in metabolism, size and shape of the midgut, the intestinal stem cells (ISCs) that replenish the intestinal epithelium of adult flies divide more in females than males. After mating the female ISCs proliferate further: the midgut undergoes extensive remodelling, changing its lipid metabolism and increasing midgut size to prepare for energy-intensive egg laying.

It has been previously reported that females produce more proliferative $Notch^{RNAi}$ and $Ras^{G12V}APC^{-/-}$ midgut tumours than males. We have confirmed this sexual dimorphism is also present in *Hippo* pathway tumours induced by activation of *Yorkie* (Yki^{3SA}) or downregulation of *Warts* (Wts^{RNAi}).

Both $Notch^{RNAi}$ and $Ras^{G12V}APC1^{RNAi}$ tumours show an enhancement in proliferation after mating in females, reflecting the mated females larger and more proliferative midguts. Unexpectedly, two genetically distinct *Hippo* pathway tumours (Yki^{3SA} and Wts^{RNAi}) instead have (i) unusually high proliferation in virgin females, and (ii) lack of enhanced proliferation in response to mating. This suggests that differences in *Hippo* signalling may normally contribute to the reproductive differences in intestinal proliferation.

This demonstrates that different midgut tumour models respond differently to sex and reproductive status. Next we will explore the underlying molecular mechanisms, focusing on the *Hippo* pathway and metabolism more broadly.

957B Imbalances in active and repressive chromatin states underlie phenotypes caused by the oncoproteins H3 K27M and EZHIP *Sam Krabbenhoft*, Tyler Masuda, Truman Do, Siddhant Jain, Peter Lewis, Melissa Harrison University of Wisconsin-Madison

Central nervous system (CNS) tumors are the leading cause of solid tumor death in children. Among the deadliest and most common pediatric brain tumors are diffuse intrinsic pontine glioma (DIPG) and posterior fossa ependymoma type A (PFA). Two key molecular events drive nearly all cases of these two cancers: a lysine-to-methionine mutation at residue 27 on histone H3 (H3 K27M) in DIPG or elevated expression of the previously uncharacterized protein EZHIP in PFA. These tumors share striking similarities, including a near-complete loss of histone H3 trimethylation at lysine 27 (H3K27me3), a mark that contributes to transcriptionally silent chromatin. We have previously shown that H3 K27M and

EZHIP are potent inhibitors of the H3K27 histone methyltransferase Polycomb repressive complex 2 (PRC2) in mammalian cell culture. Despite this, low levels of residual H3K27me3 remain at sites of initial PRC2 recruitment. These data leave open the possibility that oncogenesis is the result of transcriptional repression maintained at specific genes, aberrant activation of other genes that lose the repressive H3K27me3 mark, or a combination of both. To begin to address this question, we have modeled these cancers by expressing both oncoproteins in *Drosophila melanogaster*, the organism in which PRC2 was originally identified. Tissue-specific expression of either EZHIP or H3 K27M causes detrimental phenotypes and the loss of H3K27me3. We have leveraged these Drosophila models to screen 438 conserved, chromatin-regulatory genes for suppression or enhancement of the phenotypes. Our RNAi screen has identified over 50 genes whose knockdown modifies the phenotype caused by H3 K27M in the wing. Remarkably, these suppressors restore normal development despite the continued loss of H3K27me3. Shared features of these suppressors suggest that restoring normal development requires a precise balance between the repressive H3K27me3 and marks of active chromatin at gene regulatory elements. Ongoing studies will determine whether these emerging mechanisms can be leveraged to prevent or even reverse the phenotypes of these potent oncoproteins.

958C Targeting the Ras/Raf/ERK negative regulator *sprouty* as a novel strategy for cancer therapy *Silvia Ziliotto*, Fisun Hamaratoglu Cardiff University

Genetic mutations are the major driver of cancer development. Aberrations within the EGFR-Ras-MAPK signalling pathway are the most frequently observed in human cancer, making this pathway a good target for therapy. Another signalling pathway that is gaining increasing interest is the Hippo signalling pathway, due to its involvement in cancer. The *Drosophila* imaginal discs have been extensively used as a model to study patterns of cancer growth, as most cancers arise from the epithelial tissue. Using *Drosophila* imaginal discs, we have discovered that the output of the Ras signalling pathway in promoting proliferation versus differentiation is under the tight control of the Hippo pathway. This study identified a cluster of 26 genes that are only upregulated with simultaneous dysregulation of the EGFR and Hippo signalling pathways and are largely unaffected with a mutation in a single pathway. Here we used a Gal4/UAS system to drive Ras or Raf gain of function combined with Yki upregulation using an *apterous* driver to generate our cancer model. This leads to a dramatic overgrown phenotype of the wing imaginal disc compared to wild type discs and extends the larval developmental stage up to 9 days. We have used this model to perform an RNAi screening of the aforementioned 26 genes and have identified that downregulation of negative regulators of the EGFR signalling pathway led to a rescue of the observed overgrown phenotype. Between these screening hits, we are focusing our investigation on the gene *sprouty*. This is a negative regulator of the EGFR signalling pathway whose function as either a tumour suppressor or oncogene in cancer has shown conflicting findings. Upregulation of *sprouty 2* (the human ortholog of *Drosophila sprouty*) is common in colorectal cancer where dysregulation of both the Hippo and EGFR pathways are reported. Several studies have shown that downregulation of *sprouty 2* using siRNA led to decreased cancer proliferation and invasion. The similarity to our *in vivo* system has led us to investigate this gene further. Coupling an *in vivo* (*Drosophila melanogaster*) and *in vitro* model (colorectal cancer cell lines), we are investigating whether Sprouty can be a better target for therapy in those cancers where dysregulation of this gene could lead to a worse outcome.

959A Salt-inducible kinases synergise with Homeodomain-interacting protein kinases to promote significant tumour growth *Kewei Yu*, Nivi Ramkumar, Esther Verheyn Simon Fraser University, Burnaby, British Columbia, Canada

Homeodomain-interacting protein kinases (Hipks) are conserved kinases that regulate cell proliferation, apoptosis and tissue development. Hipks have been shown to regulate the activity of many key conserved signaling pathways, including Wnt, Notch, Hippo and Jak/Stat. Overexpression of Hipk in Drosophila causes tumorigenic phenotypes. We primarily examine tissue overgrowth and distortion in larval imaginal discs. We found that co-expression of a constitutively active form of Salt-inducible kinase 2 (Sik2) or Sik3 with Hipk caused significant tissue overgrowth and tissue distortion, indicating that both Sik2 and Sik3 can synergize with Hipk to promote tumour growth. Larvae expressing these neoplastic growths also display an extended larval phase, characteristic of *Drosophila* tumor models. SIKs are serine/threonine protein kinases belonging to the adenosine monophosphate (AMP)-activated protein kinase (AMPK) family. While mammals have SIK 1-3, *Drosophila* only has Sik2 (mammalian ortholog of SIK1 and SIK2) and Sik3. Furthermore, depletion of Sik 2 or Sik3 can suppress overgrowth phenotypes due to Hipk overexpression. Examination of total protein levels from fly tissues showed that Hipk proteins were reduced when Siks were depleted through RNAi or by using mutant alleles. These results suggest that Siks may regulate Hipk protein stability and/or activity. Furthermore, Siks are known to regulate several signal transduction cascades by phosphorylating key components of these pathways. Preliminary data from the lab has shown that Sik2 can be co-immunoprecipitated with Hipk. In summary, our research demonstrates a novel function of Siks in synergizing with Hipk to promote tumour growth potentially. We are investigating the mechanism underlying these interactions and hypothesize that Siks phosphorylate Hipks to modulate their stability and or activity. Gaining further insight into tumorigenic synergies has potential implications of finding drug targets in the future that could block tumour growth via inhibition of Siks.

960B Using optogenetic cardiac pacing and imaging to develop new heart function research platform *Elena Gracheva*, Fei Wang, Abby Matt, Hongwu Liang, Matthew Fishman, Chao Zhou Washington University in St Louis

Myocardial infarction remains the leading cause of death and myocardial ischemia contributes to 2/3rds of all cases of heart failure, which is rapidly emerging among the leading causes of mortality and morbidity in the U.S. *Drosophila melanogaster* is a simple and powerful genetic model system to investigate the role of genes associated with human diseases, including cardiac diseases. As short-lived animals, flies represent an excellent opportunity to model age or disease dependent changes in cardiac function that can be traced throughout life. The fly's heart tube is located on the dorsal side of its abdomen within 200 µm from the tissue surface allowing visible to near-infrared light to reach the heart tube. This enables non-invasive optical pacing of the fly heart using existing optogenetic tools. Morphological and rhythmic changes in a relatively simple fly heart can be readily analyzed with a non-invasive biomedical imaging technology, Optical Coherence Tomography (OCT). We developed transgenic system consisting of spatiotemporally regulated ChR2, ReaChR, and eNpHR2.0 opsins in *Drosophila*. We have demonstrated the tissue specific opsin expression and performed the heart pacing experiments. The next step will enable us to characterize changes of the fly heart function in response to different stress challenges providing insights into molecular mechanisms of ischemic preconditioning.

961C Optogenetic control of *Drosophila* cardiac function with ChRmine and ReaChR opsins *Fei Wang*, Elena Gracheva, Abby Matt, Hongwu Liang, Matthew Fishman, Chao Zhou Washington University in St. Louis, Saint Louis, MO

Cardiac optogenetics is a promising alternative to traditional electrical stimulations in controlling the activity of cardiac tissues non-invasively. In recent years, cardiac functions of animals such as rats, zebrafish, and fruit fly, have been controlled by opsin activation. In this study, we expressed red-shifted excitatory opsins (ReaChR and ChRmine) in the heart of *Drosophila melanogaster*. For ReaChR and ChRmine, we used red-light stimulation for deep penetration into the myocardial structures. Compared to existing opsins, the newly generated opsin ChRmine is able to induce spiking with much lower irradiance power. M-mode images acquired with custom optical coherence microscopy (OCM) system demonstrated controlled heart function *in vivo* and in real-time throughout the *Drosophila* life cycle (larva, early pupa, late pupa, and adult). Fast kinetics, low stimulation power, and broad heart-rate adjustable range were demonstrated for light pulses pacing. *Drosophila* heart rate can be enhanced to the frequency of the light excitation, faster than its resting heart rate, and reversible acceleration of heartbeats can be achieved at various developmental stages. This study demonstrated non-invasive cardiac control through promoting heart functions of an intact animal, which is promising for the nondestructive studies of cardiac diseases, such as congenital heart disease, posteriority bradycardia, tachycardia, and regional mechanical dys-synchrony.

962A Mitochondria malfunction and RNaseZ-associated cardiomyopathy *Ekaterina Migunova*[1], Stefania Bonanni[1], Megan Kurz[1], Alessia Vata[1], Hongwu Liang[2], Fei Wang[2], Chao Zhou[2], Edward Dubrovsky[1] 1) Fordham University, Bronx, NY; 2) Washington University, St Louis, MO

Cardiomyopathy (CM) is a disease of heart muscle leading to changes in heart wall morphology, reduced heart contractility and obstruction of blood flow. It affects people of all ages; the adult form of CM is often secondary to systemic causes such as inflammation, malnutrition and other conditions, while infantile CM occurs due to inherited mutations. Mutations of ELAC2/RNaseZ gene, which encodes RNaseZ enzyme, lead to an especially aggressive form of HCM with early onset of symptoms and lethal outcome within one year after birth. RNaseZ is a highly conserved vital enzyme with homologs in all eukaryotes. It is essential for tRNA maturation and therefore for protein synthesis. It has a dual role within a cell, as it is the only enzyme that processes 3'-ends of pre-tRNA in both nucleus and mitochondria. Previously we have generated a novel fly model of RNaseZ linked CM and demonstrated that it mimics the human disease in all major symptoms. Further investigation of the underlying mechanisms leading to RNaseZ linked abnormal heart morphology is challenged by the dual role of RNaseZ within a cell.
Here we report the effect of CM-linked RNaseZ mutations on mitochondria function. We have found that RNaseZ mutations cause a decrease in mitochondrial complex I activity, which leads to a reduction in mitochondrial ATP synthesis rates. We also have separated the nuclear and mitochondrial roles of RNaseZ to investigate the contribution of each towards the CM phenotype. We have discovered that mutating only mitochondrial RNaseZ is sufficient to cause changes in heart morphology. Lastly, we investigated whether the heart damage originating from RNaseZ dysfunction in mitochondria is cardiac specific or if it is secondary to a systemic disorder, and found that the cardiac damage associated with mutant mitochondrial RNaseZ is heart specific. Overall, these findings improve our understanding of the cellular processes contributing to RNaseZ linked CM.

963B A *Drosophila* model for human ARVC-5 caused by TMEM43^{S358L} *Nora Klinke*[1], Sandra Ratnavadivel[2], Marcel Reinhardt[1], Heiko Meyer[1], Anders Malmendal[3], Hendrik Milting[2], Achim Paululat[1] 1) University of Osnabrueck, Department of Zoology and Developmental Genetics (Germany); 2) Erich and Hanna Klessmann Institute, Heart and Diabetes Center NRW, University Hospital of the Ruhr-University Bochum, Bad Oeynhausen (Germany); 3) Roskilde University, Department of Science and Environment (Denmark)

Human *TMEM43* encodes a membrane protein with four transmembrane domains, which localizes to the ER- outer membrane compartment. A point mutation in TMEM43 results in arrhythmogenic right ventricular cardiomyopathy

(ARVC) type 5[1,2,3]. Characteristics of ARVC-5 are ventricular tachycardia, heart attacks, and sudden cardiac death[4]. Mostly, men between the ages of 20-40 years are affected. The identified point mutation is a missense mutation in the human *TMEM43* gene that results in incorporation of the amino acid leucine rather than serine at position 358 of the protein, S358L.
TMEM43 is a highly conserved protein throughout different species. Homologous proteins exist in chimpanzees, rhesus monkeys, dogs, cows, mice, frogs and fruit flies. The corresponding homolog in *Drosophila melanogaster* is CG8111. Interestingly, the serine, which is involved in the familial mutation in humans, as well as the surrounding region, are highly conserved in fruit flies (S333) and other species[5].
To investigate the effect of the point mutation in flies, we overexpressed CG8111^{S333L} ubiquitously or in a tissue specific manner. Our studies showed that ubiquitous overexpression of the mutant, but not the wild type form of CG8111, leads to lethality at pupal stages, less food uptake and increased lipid droplets in adipocytes of 3rd larvae. Furthermore, we applied mass spectrometry (MS)-based proteomics and are able to show that CG8111^{S333L} animals up-regulate proteins, which are involved in fatty acid metabolism pathways. Subsequent metabolomic analysis by nuclear magnetic resonance (NMR) spectroscopy revealed that short fatty acids accumulate in animals overexpressing CG8111^{S333L} compared to controls.
Moreover, we examined the effect of heart specific overexpression of CG8111^{S333L}. Therefore, we used the Semi-automatic Optical Heartbeat Analysis (SOHA) software to analyse heart beat parameters like heart rate, systolic and diastolic interval, fractional shortening and arrhythmicity index. We found that heart specific overexpression of CG8111^{S333L} leads to arrhythmia in five week old male flies.
In the end, we compare our findings with models for TMEM43^{S358L} in mouse and rat.

[1] Baskin et al., *Human Genetics*, 2013
[2] Christensen et al., *Clinical Genetics*, 2011
[3] Milting et al., *European Heart Journal*, 2015
[4] Fontaine et al., *Circulation*, 1998
[5] Merner et al., *The American Journal of Human Genetics*, 2008

964C New genetic avenues in Congenital Heart Disease: Ribosomal protein genes as regulators of cardiac growth (via *YAP/yorkie*) and proliferation (via *p53*) along with cardiogenic transcription factors *Tanja Nielsen*[1], Anais Kervadec[2], Xin-Xin I. Zeng[1], Analyne Schroeder[1], Jeanne L. Theis[3], Timothy M. Olson[3], Karen Ocorr[1], Paul Grossfeld[4], Alexandre R. Colas[2], Georg Vogler[1], Rolf Bodmer[1] 1) Development, Aging and Regeneration Program, Sanford Burnham Prebys Medical Discovery Institute, La Jolla, CA, USA; 2) Human Genetics Program, Sanford Burnham Prebys Medical Discovery Institute, La Jolla, CA, USA; 3) Division of Pediatric Cardiology, Department of Pediatric and Adolescent Medicine, Mayo Clinic, Rochester, MN, USA,; 4) Department of Pediatrics, UCSD School of Medicine, La Jolla, CA, USA

Hypoplastic Left Heart Syndrome (HLHS) represents the most lethal Congenital Heart Disease (CHD) and is characterized by a severely underdeveloped left ventricle. The genetic mechanisms leading to HLHS are poorly understood, but likely of oligogenic origin.
Decreasing sequencing costs have led to the identification of thousands of putative human disease variants. However, establishing genotype-phenotype relationships remains challenging. *In vivo* functional analysis of patient-derived genes and gene clusters in a high-throughput manner using the fly heart model combined with human iPSC-cardiomyocytes (hiPSC-CMs) and zebrafish, provides a framework for prioritizing and interrogating the contribution of genetic variants in heart disease.
We performed whole genome sequencing in a familial HLHS case and a cohort of 25 HLHS proband-parent trios with poor clinical outcome. Candidate genes identified by Mendelian modeling in the 25 trios were subject to gene network enrichment analysis, revealing an over-representation of ribosomal protein (RP) genes. Segregation analysis in the familial case identified a rare promoter variant affecting *RPS15A*. Remarkably, patient-derived iPSC-CM proliferation was reduced compared to the parents.
Functional analysis in model systems showed that knockdown (KD) of RPs reduced proliferation in generic hiPSC-CMs and impaired cardiac differentiation in *Drosophila* resulting in a partial or 'no' heart phenotype in adult flies. Furthermore, *RpS15Aa* KD led to cardioblast misspecification during early cardiogenesis. Functional validation in zebrafish revealed that *rps15a* KD causes reduced cardiomyocyte numbers and contractility, and defective heart looping, without affecting overall embryonic development. Strikingly, *RPS15A* KD-induced defects were significantly reversed by *p53* KD in hiPSC-CMs or zebrafish, and by *myc* KD or YAP/*yorkie* overexpression in flies, dependent on *yorkie*'s co-factor TEAD/*scalloped*.
When testing for cardiac-specific RP functions, we found synergistic interactions between *RPS15A* and cardiac transcription factor *tinman/Nkx2.7* and *dorsocross/Tbx5a*, in both *Drosophila* and zebrafish suggesting conserved synergism between RPs and cardiogenic genes in both models.
In summary, we conclude that RP genes play a critical role as regulators of cardiac growth and cardiomyocyte proliferation along with cardiogenic transcription factors and we suggest that RP genes represent a novel class of genetic effectors in CHDs, such as HLHS.

965A Characterizing Robinow Syndrome-associated DVL1 mutations in *Drosophila* *Katja MacCharles*[1], Sarah Gignac[2], Katherine Fu[2], Joy Richman[2], Esther Verheyen[1] 1) Simon Fraser University; 2) University of British Columbia

Human development is regulated by intricate, and interconnected, signal transduction networks. Given the complexity, deciphering the effects of mutations that give rise to abnormal development can be challenging. Using *Drosophila melanogaster* can simplify the puzzle of studying human disorders as flies have little genetic redundancy and are significantly easier, cheaper, and faster to raise than other vertebrate models. I use *Drosophila* to characterize Dishevelled1 (DVL1) mutations obtained from patients with Robinow Syndrome (RS). RS is a rare genetic disorder associated craniofacial abnormalities and shortened stature. Most of the mutations associated with RS affect components of the non-canonical/Planar Cell Polarity (PCP) pathway of Wnt signaling. Wnt signaling is involved in embryonic development and homeostasis. The two main pathways, canonical and non-canonical/PCP Wnt signaling, require DVL but there is still much to learn about PCP signaling which mediates cytoskeletal rearrangement events and orients cell polarity within the epithelial plane. There are 3 DVL proteins found in vertebrates, and a single ortholog, Dsh, in *Drosophila*. Each of the DVL1 variants I study have unique frameshift mutations that replace the highly conserved C-terminus with the same novel peptide sequence of no known homology. I use the Gal4-UAS system to express wildtype human DVL1 and three DVL1 variants in *Drosophila*. My research has shown that these DVL1 patient variants disrupt the stability of Armadillo/β-catenin, ectopically induce PCP/JNK signaling and activate apoptosis. Furthermore, the variants induce several novel phenotypes in wing tissue such as anterior cross vein abnormalities, ectopic bristles, and vein thickening, suggesting novel functions in other conserved signaling pathways. By understanding how conserved signaling pathways are altered by these DVL1 variants, we gain insight into the underlying mechanisms of non-canonical Wnt signaling and more broadly, how development in individuals with RS is altered. This information may guide future therapeutics for RS patients.

966B The Drosophila ortholog of POLR1D, an RNA Polymerase I & III assembly protein, is required for development *Ryan Palumbo*, Alana Belkevich, Bruce Knutson SUNY Upstate Medical University

rRNA expression and ribosome assembly are becoming appreciated as processes required for proper development. Defects in rRNA synthesis/processing, ribosomal protein expression, and ribosome assembly, all constitute a class of diseases referred to as ribosomopathies. Ribosomopathies are characterized by a tissue-specific requirement for sufficient translational output not being satisfied due to mutations in genes ultimately involved in translation. Several animal models of ribosomopathies have been established to study the molecular and physiological consequences of those mutations. *Drosophila* is emerging as an excellent model system to study ribosomopathies in various developmental contexts. Here, we establish *Drosophila* as a model to study the ribosomopathy Treacher Collins Syndrome (TCS). TCS is caused by mutations in several genes, one of which is *POLR1D*. POLR1D protein forms a heterodimer with POLR1C, which is a prerequisite to the assembly of RNA Pol I and Pol III, which synthesize 47S and 5S rRNAs, respectively. Loss of *POLR1D* function is thought to affect polymerase assembly, reduce the production of rRNAs, and impair ribosome assembly, which ultimately affects translation. We found that flies hemizygous for an allele of *Drosophila POLR1D* (*dPOLR1D*) are pre-adult lethal, and viable larvae exhibit reduced growth, and are arrested in the L1 and L2 instar stages. *dPOLR1D* mutant larvae have reduced levels of 18S and 28S rRNAs, and this is reflected in heterozygous *dPOLR1D* adults, which exhibit the *bobbed* phenotype characteristic of mutants lacking ample rRNA production. Thus, dPOLR1D is required for rRNA expression, which is necessary to complete larval development. We sequenced this allele of *dPOLR1D*, and identified a missense mutation causing a substitution in an evolutionarily conserved amino acid that has been found to be mutated to a different residue in a single case of non-familial TCS. We found that the “fly mutation” in both dPOLR1D and human POLR1D (hPOLR1D) does not affect binding of POLR1D proteins to their cognate POLR1C proteins; however, the “human mutation” does. This suggests that while different amino acid mutations can both affect development, the molecular mechanism by which this occurs can be different. TCS is a disease of neural crest cells. We performed a preliminary RNAi screen in several cells types of the nervous system, and found that as in humans, dPOLR1D is required in the nervous system to complete development.

967C A novel assay to study salivary gland dysfunction in a model of *NGLY1* deficiency John Pleinis[2], Kevin Hope[1], Danielle DaCrema[1], *Clement Chow*[1], Aylin Rodan[2] 1) Department of Human Genetics, University of Utah School of Medicine, Salt Lake City, UT; 2) Department of Internal Medicine, Division of Nephrology and Hypertension, and Molecular Medicine Program, University of Utah School of Medicine, Salt Lake City, UT

NGLY1 deficiency is a rare disorder caused by loss of function mutations in the *NGLY1* gene and often presents with developmental delay, liver dysfunction, and seizures. One of the most consistent and defining disease phenotypes is the reduced ability to make tears, sweat, and saliva. Therapies that could treat these secretory problems could have a profound effect on quality of life for these patients. To study salivary gland dysfunction in adult flies, we developed a “rock candy” assay, which measures the consumption of a solid blue sugar. Consumption, as measured by blue dye, is correlated with salivary gland function. Ablation of the salivary gland results in little to no consumption of the blue sugar. To model NGLY1-induced salivary gland dysfunction, we used RNAi to knockdown NGLY1 (*Pngl* in flies) expression in the salivary glands of adult flies and found that this reduces consumption by nearly 50%. We previously showed that

the Na-K-Cl cotransporter, NKCC1 (*Ncc69* in flies), is an enzymatic target of NGLY1 and a modifier of NGLY1 deficiency in flies. NKCC1 regulates ion flux in secretory cells like those in the salivary gland. We generated salivary gland-specific knockdown of NKCC1 and found a severe consumption defect. To test whether the defects we observed in either single knockdown was due to a genetic interaction, we generated salivary gland-specific double knockdown. The double knockdown had a severe consumption defect similar to what we observed in the single NKCC1 knockdown, suggesting that the defects observed are due to a genetic interaction between NGLY1 and NKCC1. We also demonstrate that drugs that alter NGLY1 or NKCC1 activity can rescue the consumption phenotype in NGLY1 knockdown. Together, these data indicate that the fly is a powerful model for studying salivary gland dysfunction in NGLY1 deficiency and may help develop life changing therapies.

968A *De novo* variant in *MRTF-B* is associated with intellectual disability, minor dysmorphic features, expressive language delay, impulse control issues, and fine motor delay. *Jonathan Andrews*[1], Oguz Kanca[1], David Li-Kroeger[1], Cyndi Tifft[2], Ellen Macnamara[3], Shinya Yamamoto[1], Hugo Bellen[1], May Malicdan[4], Michael Wangler[1] 1) Baylor College of Medicine; 2) NIH, Bethesda, MD; 3) NIH/NHGRI, Arlington, VA; 4) NIH/NHGRI, Rockville, MD

Myocardin-related transcription factor B (MRTF-B) 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. The MRTF-B protein 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 a proband with a *de novo* variant in the second RPEL domain of *MRTF-B* with intellectual disability, minor dysmorphic features, expressive language delay, impulse control issues, and fine motor delay. We have generated a fly model of human *MRTF-B*, using a UAS construct carrying either the human reference or variant cDNA. Expression of the UAS-human variant cDNA under the control of the *Nubbin-Gal4* driver was sufficient to induce significant morphological changes in the wing, while the expression of the human reference cDNA produced only minor changes in the posterior crossvein. Expression of *Drosophila Mrtf* using the *Nubbin-Gal4* driver produced a similar change in crossvein length as was observed with the human reference. In *Drosophila*, the SRF ortholog is known as *blistered (bs)* and is known to suppress wing vein formation and promote the development of intervein cells. As exogenous co-expression of *bs* and *Mrtf* has been previously shown to significantly alter wing morphology, we also expressed our human reference and variant cDNA lines concurrently with a UAS-*bs* line. In these experiments we found that wing morphology was highly disrupted when *bs* and the reference human cDNA were co-expressed, while the co-expression of human variant cDNA and *bs* was lethal. Based on these findings, we sought and identified two additional probands with variants within or near the second RPEL domain. Expression of these additional variants using *Nubbin*-Gal4 produced changes in the posterior crossvein analogous to the changes observed when our human reference line was expressed. Taken together, these findings suggest that different residue changes within the RPEL domain of *MRTF-B* can have drastically different morphological effects in the fly wing, and our initial *de novo* variant may underly a novel disorder.

969B Identification of gene expression changes in response to vitamin A deprivation *Deepshe Dewett*, Maryam Labaf, Khanh Lam-Kmath, Kourosh Zarringhalam, Jens Rister UMASS Boston

Chronic vitamin A deficiency (VAD) leads to blindness, delayed growth, impaired immunity and is the leading cause of preventable childhood blindness. In contrast, VAD in flies does not result in developmental defects or lethality. But similar to mammals, flies deprived of vitamin A have defects in the visual system, like damaged photoreceptors and loss of light-sensing pigments. However, a gaping hole remains in our understanding of the molecular response to VAD. To identify genes and signaling pathways that respond to VAD, we performed RNA sequencing using *Drosophila* heads. We identified 68 differentially expressed genes between vitamin A-rich and deprived conditions. Out of these, 50 genes were upregulated, and 18 genes were downregulated on VAD. Among these are the genes that are responsible for the synthesis of the retinal chromophore (*ninaB, ninaG, Pdh*) and the termination of the phototransduction pathway (*Arr1, Arr2, inaC, stops*). We also detected significant changes in the expression of genes that encode for specific aminoacyl-tRNA synthetases (*GluProRS, IleRS, LeuRS, LysRS*), major nutrient reservoir proteins (*Lsp1alpha, Lsp1beta, and Lsp2*), calcium buffers (*Cpn, Cnx99a*), and factors that mediate stress or immune responses (*per, Cyp309a1, TotA, TotC, TotM, dnr1, Diedel*).
A comparison between genes responding to VAD and previously identified blue light stress-responsive genes showed a largely nonoverlapping transcriptome response to these two types of environmental stresses. Only seven genes, with poorly understood functions, were similar between these two studies. Taken together, our study provides insights into the molecular mechanisms that respond to vitamin A deprivation.

970C Transcriptomic analysis in NF1: exploring drivers of diverse phenotypes *Connor N. Broyles*, Valentina Botero, Seth M. Tomchik Department of Neuroscience, The Scripps Research Institute, Scripps Florida, Jupiter, FL, USA.

Neurofibromatosis type 1 (NF1) is a chronic multisystem genetic disorder affecting approximately 1 in 3,500 humans. NF1 patients often experience multiple symptoms such as tumors, cognitive dysfunction, sleep disturbances, and metabolic changes. NF1 is caused by mutations in the *NF1* gene, which encodes a protein called neurofibromin. One of the major

biochemical functions of neurofibromin is Ras GAP activity, which downregulates Ras signaling. Loss of function of the catalytic neurofibromin GAP-related domain, which is responsible for neurofibromin's Ras GAP activity, is a major contributor to NF1 phenotypes such as increased energy expenditure. In addition, Ras-independent effects on other signaling pathways, such as cAMP/PKA, have been described for phenotypes like short stature and sleep disruption. The impacts on relatively pleiotropic cellular signaling pathways, as well as the systemic interactions among different cell types, raises the question of how loss of neurofibromin drives systemic and organismal pathophysiology in NF1. Downstream molecular pathways remain elusive. This study aims to identify key pathways involved in NF1 pathology using a transcriptomics approach.

Loss of neurofibromin may drive effects on organismal physiology via alterations in the transcription of downstream gene products. To gain insight into the transcriptional landscape in NF1, we have examined the effects of loss of neurofibromin on gene expression in *Drosophila*. RNA sequencing was used to identify differentially expressed genes (DEGs) in Nf1 mutants ($NF1^{P1}$) as compared to wild-type controls (w^{CS10}). RNA from heads and bodies was compared between mutants and controls to identify DEGs in neuronally-enriched samples vs. the rest of the body. Analyses revealed several classes of dysregulated transcripts in NF1 mutants; specific for heads/bodies or shared in both. Gene ontology analysis revealed several enriched processes and pathways in NF1 mutants, with association to known human phenotypes, and follow-up genetic experiments are narrowing key pathways for NF1 effects on organismal phenotypes.

971A CryAB is a target protein of NUAK kinase activity to prevent protein aggregation in muscle tissue *Ziwei Zhao*, Erika Geisbrecht 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 cell polarity or cell motility by controlling the assembly and/or disassembly of cytoskeletal proteins. Previous results in our lab have demonstrated that a mutation in *Drosophila* NUAK results in the degeneration of larval body wall muscles. One prominent feature in these muscles is the abnormal accumulation of select proteins, which displaces other components of the contractile sarcomere apparatus. To better understand how kinase activity contributes to NUAK function, we mutated important residues in the conserved kinase domain using CRISPR/Cas9 and transgenic mutagenesis approaches. Indeed, mutation of a key catalytic residue within the kinase domain resulted in earlier lethality and thinner muscles. These results were confirmed by expressing a sallimus (Sls)-GFP fusion protein to visualize the dynamics of this process in vivo. Next we wanted to determine if we could separate the requirement for NUAK in embryonic muscle development from protein aggregation and muscle degeneration in larval contractile muscles. Compromising NUAK kinase activity in larval muscle using a post-embryonic driver still resulted in protein aggregation, thus suggesting that NUAK has independent functions in muscle development and muscle homeostasis. We have confirmed that a conserved threonine residue in the kinase domain of NUAK is essential to promote protein activity. To uncover proteins that may be substrates of NUAK kinase activity, 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. Taken together these data suggest that CryAB may be a target of NUAK phosphorylation in the prevention of protein aggregation.

972B Survival and motility of adult *Drosophila melanogaster* flies fed high-calorie diets during early development Mario Cayetano-Velazquez[1], Irma Dueñas-García[1], Luis Felipe Santos-Cruz [1], Laura Castañeda-Partida[1], Evelyn Hernández-Torres [1], Elizabeth Soto-López[1], Daniela Vargas-Sánchez [1], Nefte Meni Yañez-Mendoza[1], María Eugenia Heres-Pulido[1], *Noma Velazquez-Ulloa*[2] 1) Lab. de Genética Toxicológica, FES Iztacala UNAM. Av. Los Barrios # 1. Los Reyes Iztacala, Tlalnepantla, Estado de México, CP 54090. ; 2) Lewis & Clark College

Excessive consumption of some macronutrients, such as lipids and carbohydrates can contribute to development of cardiovascular disease, neurodegenerative disease, obesity, and metabolic syndrome, among others. *Drosophila melanogaster* is an effective model organism to determine the effects of the consumption of hypercaloric diets during development because of the high conservation in the metabolic and signaling pathways between mammals and insects, in which many of the participating genes have orthologs in *Drosophila.* We evaluated the effect of different diets on survival and negative geotaxis in two *Drosophila* strains, Canton-S (CS, wildtype), and Oregon R(R)-flare (OR), which have increased Cyp450 enzyme levels and have been shown to be insecticide resistant. Larvae from these fly strains fed on either a Normal Diet (ND), a High Fructose Diet (FR; 17% fructose), a High Palmitic Acid Diet (AP, 3.2% palmitic acid), or a Mixed Diet (MX, 5% FR + 1% AP) until pupation. For the survival assays, ten recently emerged adult flies per vial, per treatment, per sex, per fly strain, were transferred to vials with ND food. Three vials per condition were collected per experiment, and five independent experiments were conducted. Flies were transferred to new vials and the number of survivors per vial was counted every 3 days for 54 days. To determine effects on survival, we estimated mean survival, time to 50% survival, maximum survival and performed a Kaplan-Meier survival analysis. We found that survival was significantly decreased by the hypercaloric diets. In the CS flies, all 3 high calorie diets decreased survival, while OR

flies had decreased survival with the FR and AP diets, but not in the MX diet. In addition, the magnitude of the effect of the diets on survival was greater in the CS strain than in the OR strain, which suggests that increased P450 enzyme levels may confer protection. Next, we evaluated the effect of the different diets on negative geotaxis. For this assay, ten recently emerged flies per vial, per treatment, per sex, per fly strain, were transferred to vials and tested on a climbing assay. CS flies from the high calorie FR diet climbed the most, while OR flies that consumed the ND climbed the least. The high calorie treatments affect female and male flies of the CS and OR fly strains differently. We also noticed differences between these two strains, which support an involvement of the xenobiotic metabolism in the effects of high caloric diets

973C Genotype-by-Sex-by-Exercise Studies Using *Drosophila melanogaster*: Comparing the Power Tower and the TreadWheel as Two Exercise Apparatuses *Tolulope Kolapo*[1], Jordan Albrecht[2], Michelle Tan[3], Mckenzie Chamberlain[4], Annie Backlund[5], Alyssa Koehler[6], Sean Shelley Tremblay[7] 1) The University of Alabama, Tuscaloosa, AL; 2) The University of Alabama, Tuscaloosa, AL; 3) The University of Alabama, Tuscaloosa, AL; 4) The University of Alabama, Tuscaloosa, AL; 5) The University of Alabama, Tuscaloosa, AL; 6) The University of Alabama, Tuscaloosa, AL; 7) The University of Alabama, Tuscaloosa, AL

Exercise is a cost effective intervention strategy that has been recognized to play a role in prevention and corrective response to Metabolic Syndrome (MetS). MetS is a combination of metabolic disorders that are increasingly prevalent in Western cultures. Metabolic disorders associated with MetS include elevated blood pressure, increased blood-sugar levels, abnormal cholesterol and increased triglyceride concentrations. To determine the effect of exercise on obesity and other metabolic disorders, daily exercise regimes can be simulated using Drosophila melanogaster. The negative geotaxis; an innate escape response where flies ascend the wall of a cylinder when tapped to the bottom is a characteristic climbing tendency of the fruit fly that can be taken advantage of to induce exercise. The TreadWheel and Power Tower are two apparatuses that have been used to induce exercise in flies under controlled settings. For both pieces of equipment, significant effects of exercise on diet, weight, climbing ability, aging and other phenotypes have been reported in fruit flies. Although both machines allow for regulated induction of exercise, each one provides its own set of distinct advantages and limitations. Currently, no direct comparison of the effectiveness of the two pieces of equipment has been conducted. It is therefore unclear whether the two devices differ in their ability to induce exercise or in their impact on metabolic phenotypes. Our project compares stress levels and metabolic response induced in flies by the two apparatuses, and also determines the most effective apparatus to achieve optimum exercise in adult flies by evaluating genetic and sex-specific interactions driving variation in metabolic phenotypes. This study determines the response of seven wild derived genetic lines from the Drosophila Genetic Reference Panel to exercise treatment on both apparatuses.Fitness traits such as longevity, fecundity and climbing speed are measured. Our results show that flies exercised on both apparatuses varied in their response by genotypes and sex, and flies exercised on the Treadwheel lived longer after exercise than flies exercised on the Power Tower.

974A Pupation as a critical hypoxia-sensitive stage in *Drosophila melanogaster* *Tsering Stobdan*, Nicholas Wen, Dan Zhou, Gabriel Haddad University of California San Diego

Hypoxia plays a critical role in multiple disease conditions. The highly conserved cellular and molecular mechanisms between flies and higher eukaryotes, including humans, have made *Drosophila* a valuable organism to identify hypoxia-sensitive cells and genes critical to hypoxic environment. Since t*he life cycle of Drosophila* constitutes four morphologically distinct developmental stages, a stage specific identification of mechanisms involving hypoxia response becomes critical. Although the effect of hypoxia on the embryo, larvae or adult is frequently reported, the effect on pupae or pupation is less clear. In this study, we examined different stages of *Drosophila* development for its hypoxia sensitivity and identified pupae as a critical stage affecting their subsequent eclosion. We show that the continuous exposure to 4% O_2, from embryo to pupae is lethal. However, returning to normoxia at early, mid and late pupae stage would results in 99.6%, 66.1% and 54.4% eclosion, respectively. Similarly, returning to normoxia at 1st, 2nd or 3rd instar larvae resulted in >97% eclosion. In contrast, exposing the normoxia-grown larvae or pupae to 4% O_2 is lethal. Additionally, exposing early pupae for 3 days of 4% O_2 led to only 6.1% eclosion, while shorter exposure such as for 2 days or 1 day to 4% O_2 resulted in significantly higher eclosion of 66.7% and 96.4%. These results indicate that pupation is a very sensitive stage to hypoxia during *Drosophila* development. Overall, these results will be the basis for the identification of critical genes involved in hypoxia sensitivity or tolerance during development.

975B Gapvd1 regulates slit diaphragm formation in *Drosophila* but is otherwise dispensable for fly development. Konrad Lang, *Helena Heinkele*, Julian Milosavljevic, Lea Gerstner, Tobias Hermle Renal Division, Faculty of Medicine and Medical Center, University of Freiburg, Freiburg, Germany

Introduction:
Mutations in the gene *GAPVD1* cause nephrotic syndrome in humans. GAPVD1 interacts with the early endosomal regulator RAB5 but the subcellular localization of GAPVD1 remains unclear. Silencing of *Gapvd1* in the podocyte-like *Drosophila* nephrocytes resulted in mistrafficking of fly nephrin.

Methods: We generated conditional knockdowns and a stable genetic deletion of *Drosophila Gapvd1* by CRISPR/Cas9 and used microhomology-mediated end joing to introduce a c-terminal HA-tag into the genomic locus of *Gapvd1*. We performed a functional analysis of the novel fly models.
Results:
Using coexpression of tandem *Gapvd1*-gRNAs and Cas9 in the germline we generated twin frameshift mutations at the second and third exons of the *Drosophila Gavpd1* gene. Flies carrying these mutations were homozygous viable without any overt phenotype. In contrast, the podocyte-like nephrocytes of these animals showed a severely altered slit diaphragm architecture with mislocalization of fly nephrin and the orthologue of NEPH1 as well as a partial loss of both proteins from the surface. This phenotype was similar but considerably stronger than the phenotype observed when using RNAi-mediated silencing. This suggests that the homozygous frameshift mutations result in a null allele. The phenotype was confirmed by conditional CRISPR/Cas-mediated knock down using two independent gRNAs. Deletion of *Gapvd1* in the *Drosophila* model thus results in a phenotype that manifests exclusively in podocyte-like nephrocytes. This recapitulates the disease manifestation in human patients with *GAPVD1* mutations who presented exclusivey with nephrotic syndrome, supporting a role for *Drosophila* as a model for this genetic disease. To study the subcellular localization of *Drosophila* Gapvd1, we introduced an HA-tag into the c-terminus of the Gapvd1 locus. Immunofluorescence of nephrocytes derived from the resultant knock-in lines showed co-localization of the Gapvd1-HA protein with *Drosphila* Rab5, supporting that *Gapvd1* primarily resides in early endosomes. We overexpressed human *GAPVD1* in nephrocytes, that equally localized in early endosomes. Gain-of-function of the human gene entailed reduced tracer endocytosis in nephrocytes, suggesting a dominant negative effect.
Conclusion: We established suitable new *Drosophila* models to study the function of Gapvd1 in nephrocytes as an invertebrate podocyte model and observed colocalization with Rab5 and a nephrocyte-restricted loss-of-function phenotype.

976C An *in vivo* screen identifies small molecule modulators of the endoplasmic reticulum stress response *Kevin Hope*, Alexys Berman, Clement Chow Human Genetics, University of Utah, Salt Lake City, UT

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, and 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 findings on whether 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.

977A Humanized *Drosophila* model of the Meier-Gorlin syndrome. Maxim Balasov, Katarina Akhmetova, *Igor Chesnokov* UAB

Meier-Gorlin syndrome (MGS) is a rare autosomal recessive disorder characterized by microtia, primordial dwarfism, small ears and skeletal abnormalities. Patients with MGS often carry mutations in the genes encoding the subunits of the Origin Recognition Complex (ORC), components of the pre-replicative complex (pre-RC) and replication machinery. Orc6 is an important component of ORC and has functions in both DNA replication and cytokinesis. A mutation in conserved C-terminal motif of Orc6, Y225 to S, is associated with MGS and impedes the interaction of Orc6 with the core ORC. Recently, a new mutation in Orc6, K23 to E, was identified however, it is localized in the N-terminal domain of the protein. In order to study the functions of new Orc6 mutation in live animal system we used human Orc6 gene or a hybrid Orc6-HD transgene (containing intact human N-terminal TFIIB like domain and *Drosophila* C-terminus) to rescue the *orc6* deletion in *Drosophila*. The obtained flies survive to the adult stage and allow studies of MGS mutations in humanized animal model system. Using this approach we discovered that unlike previously identified Y225S MGS mutation in Orc6, the K23 to E substitution in the N-terminal TFIIB-like domain does not disrupt the interaction between Orc6 and the rest of the ORC. However, K23E MGS mutation results in a reduced DNA binding ability of the Orc6 protein.

The flies carrying MGS mutations are unable to fly and display growth and development defects. Overall, despite having different underlying molecular mechanisms both MGS mutations resulted in similar phenotypes, deficient pre-RC formation and reduced DNA replication.
Our studies revealed the importance of evolutionarily conserved and variable domains of Orc6 protein and allowed the studies of human protein functions and the analysis of the critical amino acids in live animal heterologous system as well as provided novel insights into the mechanisms underlying MGS pathology. We believe that hybrid approach not only open a broad avenue to study new Orc6 mutations for medical and general science purposes but might be useful in other humanized models.

978B Mimicking human disease-causing mutations in Drosophila PLC-γ Mariah Torcivia, Alli Raymond, *Justin Thackeray* Clark University

Activating mutations of both PLC-γ1 and -γ2 are observed in a variety of human diseases. This includes somatic mutations in a variety of cancers, as well as germline changes underlying auto-immune disorders; some inherited, activating, PLC-γ mutations have also been shown to be protective in early-onset Alzheimer disease. Drosophila has a single PLC-γ homolog encoded by *small wing* (*sl*) and although many *sl* mutations have been isolated, all are either hypomorphic or loss of function alleles. We are therefore using CRISPR to recreate in Drosophila some of the most commonly observed mammalian PLC-γ activating mutations. These mutant lines should be useful both in understanding the role of increased PLC-γ signaling in human disease, as well as providing reagents to screen for drugs that block this activation. One successful edit made to date is viable, mimicking the PLCγ1 S345F mutation observed in the malignancies from many human T cell leukemia patients. Flies homozygous for an sl^{S349F} edited X-chromosome typically show normal eye development but, in a background with increased dosage of sl^{+}, ommatidia in two independent lines frequently show abnormal photoreceptor development, in which R3 is replaced by two photoreceptor cells with smaller rhabdomeres. This phenotype is similar to that seen in loss of function mutations of the nuclear receptor encoded by *seven-up*, in which R1, R3, R4, or R6 cells are transformed into R7. We are in the process of isolating additional activating mutations, and further characterizing the defect in the sl^{S349F}mutant line.

979C Understanding the Progressive Loss of Larval Muscle Fibers in Cachexia Tumor Model System with Focus on Myosin *Ellen Thompson*, Grace Stegemoller, Logan McDowell, Mardelle Atkins Sam Houston State University

Cachexia is a wasting syndrome common in late-stage cancer patients, whose symptoms include loss of body fat, weight loss, and muscle weakness/deterioration. To study the cachexic effects on muscles, our lab utilizes a *Drosophila melanogaster* tumor model which displays the syndrome's major symptoms. These tumor larvae fail to pupate and display observable physical wasting and symptoms of muscle loss over the period of 8-12 days AEL. In my project, I am distinguishing how the muscle sarcomere structure is changing due to cachexia. I observe a progressive loss of larval skeletal muscle strength was detailed over a five-day span (day 8-12 in development). I determined that there is a progressive loss of the larval skeletal muscle integrity, with late day 12 larvae having many broken muscle fibers. At a subcellular level, loss of sarcomere structure is increasingly common over the progression of the disease. Using immunohistochemistry, I observed that larval skeletal muscle fibers displayed major defects in actin, alpha-actinin, and myosin heavy chain localization towards the later stages of the disease, with fewer disruptions on day 8 or 10. I also observed that of these three molecules, myosin heavy chain shows the earliest and most pervasive phenotypes. From this, it is prophesied that this structural change in the sarcomere protein structure is related to the cause of the breakdown in the larval skeletal muscles. These results suggest that myosin heavy chain mislocalization is an early event in the loss of muscle function and integrity observed in these larvae, and our future studies will investigate if this is due to decreased protein production or increased turnover.

980A miR-277 targets *hid* to ameliorate Aβ42-mediated neurodegeneration in *Drosophila* eye model of Alzheimer's Disease *Prajakta Deshpande*[1], Chao-Yi Chen[2], Anuradha Chimata[1], Catherine Yeates[1], Chun-Hong Chen[2,3], Madhuri Kango-Singh[1,4,5,6], Amit Singh[1,4,5,6,7] 1) Department of Biology, University of Dayton, Dayton, OH; 2) Institution of Molecular and Cellular Biology, National Taiwan University, Taipei, Taiwan; 3) National Institute of Infectious Diseases and Vaccinology, National Health Research Institutes, Miaoli, Taiwan; 4) Premedical Program, University of Dayton, Dayton, OH; 5) Center for Tissue Regeneration & Engineering (TREND), University of Dayton, Dayton, OH; 6) Integrative Science and Engineering (ISE), University of Dayton, Dayton, OH; 7) Center for Genomic Advocacy (TCGA), Indiana State University, Terre Haute, IN

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. Furthermore, we have identified *head involution defective* (*hid*) as one of the targets of miR-277 by Fly TargetScan and validated by luciferase assay and qPCR. The *hid* transcript levels are decreased by~ 2.3-fold when miR-277 is misexpressed in the GMR>Aβ42 background in comparison to the GMR>Aβ42 fly model. Hence, here we provide a mechanism of how miR-277 modulates Aβ42-mediated neurodegeneration by regulating *hid* transcript levels and demonstrate its neuroprotective role in Aβ42-mediated neuropathology.

981B A Screen to Identify Genetic Modifiers of Seizure Susceptibility in a *Drosophila* model of *PIGA* Deficiency *Shayna Scott*, Emerald Lane , Emily Coelho, Clement Chow University of Utah, Salt Lake City, UT

Phosphatidylinositol glycan class A (*PIGA*) deficiency is an X-linked condition classified as a congenital disorder of glycosylation (CDG). CDGs are rare genetic diseases that result from 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 transferring a N-acetylglucosamine to phosphatidylinositol. 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 and rare diseases, 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 a large number of 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 associated with PIGA deficiency is highly dependent on genetic background, with seizure susceptibility between 0-60%. We also found that median seizure recovery time was also highly dependent on genetic background. There is a moderate correlation between seizure susceptibility and recovery time, suggesting only a partial overlap between the genetic architecture that underlies variability of either phenotype. We also found that there was a moderate correlation between the sexes for both phenotypes, suggesting some sex-specificity to the modifier genes. To identify modifiers of these phenotypes, we performed genome wide association analyses for each independently, and by sex. We will present functional characterization of the strongest genetic modifiers. Further investigation into genetic modifiers that impact the seizure susceptibility will provide insights into improved treatments and personalized therapies.

982C Exploring the role of *shaggy* and *dmyc* in development of combination therapy against human neuronal tauopathies in *Drosophila* *. Pragati*, Surajit Sarkar Department of Genetics, University of Delhi, South Campus, Dhaula Kuan, New Delhi, India

Human neuronal tauopathies which include Alzheimer's, Parkinson's, Pick's disease(s) etc. are group of neurodegenerative disorders characterised by aberrant tau hyperphosphorylation resulting in the formation of toxic tau oligomers, paired helical filaments (PHFs) and neurofibrillary tangles. These toxic aggregates were found in different specific regions of the brain resulting in various symptomatic features in patients. Even though several attempts are being made, the definite cause of these disorders remains unexplained certainly due to complex disease traits and limitations associated with the availability of human disease samples. We have reported earlier that targeted reduction of *dmyc* (a *Drosophila* homologue of *cmyc* proto-oncogene) constrains NFTs mediated tau pathogenesis. Further, in order to unravel the molecular insights, our findings suggest a pivotal role of *shaggy* (a *Drosophila* homologue of *gsk3β*, kinase) in conferring the *dmyc* mediated rescue against tauopathies. We observed that concurrent downregulation of both *dmyc* and *shaggy* confers an additive rescue in restricting tau hyperphosphorylation levels, neurofibrillary tangles formation, and in restoring heterochromatin loss to the physiological levels against tau-mediated toxicity in *Drosophila*. The subsequent analysis also showed that *dmyc* regulates *shaggy* activity via regulating protein phosphatase 2A (dPP2A) in a dose-dependent manner. Taken together, our study provides novel molecular insights about the role of *shaggy* in tau aetiology, which may aid in developing combinatorial drug(s) against the devastating human neuronal tauopathies.
Keywords: *Drosophila*; Tauopathies; dMyc; Shaggy; Neurofibrillary tangles

983A Coevolution is pervasive between unrelated glycosylation pathways and points to potential disease modifiers *Holly Thorpe*, Nathan Clark, Clement Chow University of Utah Department of Human Genetics, Salt Lake City, Utah

Glycosylation is one of the most common post-translational modifications. Defects in glycan biogenesis pathways, such as N-linked glycosylation, O-linked glycosylation, and GPI anchor synthesis, lead to rare, multi-systemic disorders classified as Congenital Disorders of Glycosylation (CDG). CDGs typically present with seizures, hypotonia, and developmental delay, but display large clinical variability with symptoms affecting every system in the body. This variability suggests modifier genes affect the phenotypes. I am employing evolutionary approaches to identify modifier genes of CDGs. Evolutionary Rate Covariation (ERC) relies on the premise that proteins that interact physically or genetically or are functionally related coevolve at similar rates. ERC values are calculated using the correlation coefficient of evolutionary

rates of gene pairs in a species tree. I used ERC values to look genome wide for coevolution with CDG genes, specifically genes involved in GPI anchor synthesis.
There was enriched coevolution among GPI anchor synthesis proteins. Unexpectedly, there was also enriched coevolution between GPI anchor synthesis proteins and proteins in other glycosylation pathways, suggesting more overlap between the different pathways than appreciated. Gene Ontology analysis of top genes that coevolve with GPI anchor synthesis proteins showed enrichment in genes involved in RNA modification and mitochondrial gene expression, suggesting interactions between these processes and GPI anchor synthesis. Gene pairs with the highest coevolutionary scores included both *HTT* and *PIGG* and *ATG7* and *PIGG*. *HTT* and *ATG7* are associated with neurodegenerative disorders possible indicating overlap in pathophysiology between the disorders.
To functionally validate these exciting signals, I screened for genetic interactions using the *Drosophila* eye. Many GPI anchor synthesis genes are necessary for *Drosophila* eye development and knockdown of these genes leads to rough and disorganized eyes. By creating double knockdowns of GPI anchor synthesis genes and coevolving genes in the *Drosophila* eye, I identified genetic interactions between genes previously thought to be unrelated. Many of the strongest evolutionary signals validate as interactors in this *in vivo* analysis. Coevolution is an underutilized tool for identifying interactions between unrelated proteins. These connections could lead to better understanding of glycosylation pathways and potential treatments for CDGs.

984B *Drosophila* eye model to study the role of NAT9 in Alzheimer's Disease related Dementia (ADRD) Emily Snider[1], *Prajakta Deshpande*[1], Amit Singh[1,2,3,4,5] 1) Department of Biology, University of Dayton, Dayton, OH; 2) Premedical Program, University of Dayton, Dayton, OH; 3) Center for Tissue Regeneration & Engineering (TREND), University of Dayton, Dayton, OH; 4) Integrative Science and Engineering (ISE), University of Dayton, Dayton, OH; 5) Center for Genomic Advocacy (TCGA), Indiana State University, Terre Haute, IN

Alzheimer's Disease (AD), an age-related progressive form of dementia, is characterized by a decline in cognitive function. Accumulation of the peptide amyloid beta (Aβ42) plaque is one of the characteristics of the disease. The accumulation of these Aβ42 plaques trigger the hyperphosphorylation of tau, a microtubule associated protein, thereby destabilizing the microtubules. This results in the intracellular accumulation of neurofibrillary tangles. We employed the Gal4/UAS system in *Drosophila melanogaster* to misexpress human Aβ42 within the developing fly retina, exhibiting AD-like neuropathology. Accumulation of Aβ42 plaque(s) triggers the aberrant activation of signaling pathways like the JNK pathway resulting in neuronal cell death by unknown mechanism(s). Using forward genetic screening, we identified *N-acetyltransferase 9* (*NAT9*) as one of the genetic modifiers of GMR>Aβ42 reduced eye phenotype. The previous study suggests that NAT9 stabilizes microtubules by acetylation of tubulins, thereby inhibiting JNK signaling. This study aims to understand the role of NAT9 in Aβ42-mediated neurodegeneration. The gain-of-function of *NAT9* in GMR>Aβ42 background suppresses the Aβ42-mediated neurodegeneration whereas loss-of-function of *NAT9* in GMR>Aβ42 background enhances Aβ42-mediated neurodegeneration. The eye antennal imaginal discs of loss-of-function of *NAT9* in GMR>Aβ42 background shows the activation of JNK pathway by increased pJNK levels. Hence, here we propose that NAT9 downregulates JNK signaling pathway which can ameliorate Aβ42-mediated neurodegeneration.

985V Age-dependent Lamin Remodeling Induces Cardiac Dysfunction via Dysregulation of Cardiac Transcriptional Programs *Natalie Kirkland*[1], Alexander Whitehead[1], James Hocker[1], Pranjali Beri[1], Geo Vogler[2], Bill Hum[2], Mingyi Wang[3], Edward Lakatta[3], Bing Ren[1], Rolf Bodmer[2], Adam Engler[1] 1) University of California, San Diego; 2) Sanford Burnham Prebys Medical Discovery Institute; 3) National Institute on Aging

As we age, structural changes contribute to progressive decline in organ function, which in the heart acts through poorly characterized mechanisms. Utilizing the rapidly aging fruit fly model with its significant homology to the human cardiac proteome, we found that cardiomyocytes exhibit progressive loss of Lamin C (mammalian Lamin A/C homologue) with age. Unlike other tissues and laminopathies, we observe decreasing nuclear size, while nuclear stiffness increases. Premature genetic reduction of Lamin C phenocopies aging's effects on the nucleus, and subsequently decreases heart contractility and sarcomere organization. Surprisingly, Lamin C reduction downregulates myogenic transcription factors and cytoskeletal regulators, possibly via reduced chromatin accessibility. Subsequently, we find an adult-specific role for cardiac transcription factors and show that maintenance of Lamin C sustains their expression and prevents age-dependent cardiac decline. Our findings are conserved in aged non-human primates and mice, demonstrating age-dependent nuclear remodeling is a major mechanism contributing to cardiac dysfunction.

986V Loss of MECR, an enzyme for mitochondrial fatty acid synthesis, causes iron accumulation, upregulation of ceramides and neurodegeneration *Debdeep Dutta*[1,2], Oguz Kanca[1,2], Seul Kee Byeon[3], Jun Hyoung Park[1], Paul C. Marcogliese[1,2], Zhongyuan Zuo[1,2], Rishi V. Shridharan[1], Guang Lin[1,2], Matthew Wheeler[4], Benny A. Kaipparettu[1], Akhilesh Pandey[3], Hugo J. Bellen[1,2,5], Undiagnosed Diseases Network 1) Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 2) Jan and Dan Duncan Neurological Research Institute, Texas Children's Hospital, Houston, TX; 3) Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN, US; 4) Division of Cardiovascular Medicine, Stanford University School of Medicine, Stanford, CA; 5) Department of Neuroscience, Baylor College of Medicine, Houston, TX

Fatty acid synthesis occurs in the cytoplasm as well as in the mitochondria of eukaryotic cells. Autosomal recessive mutations in *MECR* (Mitochondrial Enoyl CoA Reductase), which encodes an enzyme required for the last step of the mitochondrial fatty acid synthesis, cause a childhood-onset pediatric neurodegenerative disorder called MEPAN syndrome (MIM # 617282). To study the mechanism underlying the neurodegenerative phenotypes observed in MEPAN syndrome, we generated a severe loss-of-function CRIMIC (SA-T2AGAL4-polyA) allele of the fly ortholog of *MECR*, CG16935/*mecr* using CRISPR. Homozygous mutants die at larval stages and the lethality is rescued upon expression of the reference human *MECR* cDNA, showing functional conservation across species. Given the lethality, we performed RNAi-mediated knockdown experiments in neurons and observed progressive locomotor defects and vision loss. Upon successfully modelling the key disease phenotypes in our fly model, we used the fly and fibroblasts from patients to evaluate the mitochondrial function and morphology. A GFP-tagged allele of *mecr* expressed at the endogenous levels showed that the protein is localized to mitochondria. Transmission Electron Microscopy of Drosophila eye mutant clones and the MEPAN patient fibroblasts revealed reduced mitochondrial numbers and abnormal mitochondrial morphology. Additionally, mitochondrial function (mitochondrial membrane potential, ATP production, electron transport chain activity, and oxygen consumption rates) are all compromised due to loss of *mecr*/*MECR*. Lipidomic analyses of fly mutants and MEPAN patient fibroblasts revealed elevated ceramide levels. Hence, our next question was: how does loss of *mecr* increase ceramide levels? We found that loss of *mecr* affects the stability of mtACP, a multifunctional protein that helps in the assembly of Fe-S cluster biogenesis. Consistent with decreased mtACP levels, loss of *mecr* causes Fe-S cluster biogenesis defects and elevated iron levels in the larval brain. Excess iron results in the accumulation of ceramides leading to mitochondrial defects and neurodegeneration in MEPAN syndrome.

987V Transcription related proteins modify TDP-43 mediated toxicity in a fly model of ALS *Deepak Chhangani*[1], Swapnil Pandey[1], Lorena de Mena[1], Pedro Fernandez-Funez[1], Diego Rincon Limas[1, 2] 1) Department of Neurology, University of Florida, Gainesville, FL; 2) Department of Neuroscience, University of Florida, Gainesville, FL

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. We anticipate that modifying or altering these genes 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.

988V *Vexed* mediates non-cell autonomous loss of dopaminergic neurons *Jacinta Davis*, Elizabeth Kolaski, Daniel Babcock Lehigh University

Parkinson's Disease (PD) is the most prevalent movement disorder with about 10 million people worldwide affected. The hallmark of PD is the loss of dopaminergic (DA) neurons. Little is known as to why these neurons are vulnerable to PD. We performed a genome-wide screen using over 200 wild-caught *Drosophila melanogaster* lines from the Drosophila Genetic Reference Panel (DGRP) to identify genes involved in the maintenance of DA neurons. From this screen, we found the gene *CG42339*. *CG42339* is a previously unannotated gene with a human ortholog. Mutations and non-cell autonomous knockdown of *CG42339* caused DA neuron loss in the *Drosophila* brain at late adulthood. We also discovered a progressive locomotor defect in the mutations and knockdown of *CG42339*. We further examined *CG42339* by investigating the mechanistic function this gene plays in the maintenance of DA neurons. We were able to rescue this loss of DA neurons in a *CG42339* mutant by limiting the innate immune response and inhibiting nitric oxide signaling. *CG42339* appears to be acting as a receptor for advanced glycation end-products (RAGE), therefore we named this gene *vexed*.

989V Identification of human genes that modify concurrent Aβ42 and tau pathology in a fly model of Alzheimer's disease *Vanlalrinchhani Varte*, Jeremy W Munkelwitz, Diego E. Rincon-Limas Department of Neurology, University of Florida, Gainesville, FL, USA

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.

990V Intra- and extra-cellular functions of ALS-related ER protein VAP in *Drosophila* *Kosuke Kamemura*[1], Misako Okumura[1,2], Takahiro Chihara[1,2] 1) Program of Biomedical Science, Graduate School of Integrated Sciences for Life, Hiroshima University, 1-3-1 Kagamiyama, Higashi-Hiroshima, Hiroshima 739-8526, Japan; 2) Program of Basic Biology, Graduate School of Integrated Sciences for Life, Hiroshima University, 1-3-1 Kagamiyama, Higashi-Hiroshima, Hiroshima 739-8526, Japan

VAP is a type II integral transmembrane protein localized at the endoplasmic reticulum (ER), and functions as a tethering protein of the membrane contact sites between ER and various intracellular organelles. Recent studies revealed that VAP is cleaved, and its N-terminal MSP domain is secreted to the extracellular space in *C. elegans*, *Drosophila* and also in human. The secreted VAP MSP domain is known to bind to a variety of axon growth cone guidance receptors (Eph, Robo, Lar etc.), implying that VAP could function non-cell autonomously. In addition, mutations in human VAPB were known to cause neurodegenerative diseases such as amyotrophic lateral sclerosis 8 (ALS8). However, as the VAP loss-of-function exhibits severe lethality in early developmental stages, the physiological functions of VAP, especially the extracellular functions of VAP MSP domain are not well understood.
In order to investigate the physiological functions of VAP (*Drosophila* ortholog is Vap33), we utilized *Drosophila* olfactory projection neurons with a mosaic analysis with a repressible cell marker (MARCM), which allows us to analyze the function of a gene of interest in a single cell resolution *in vivo*. In *vap33*$^{-/-}$ MARCM clones, the dendrite but not axon of projection neurons exhibited the severe morphological defects, which are rescued by the cell-autonomous expression of *vap33* cDNA, suggesting that VAP cell-autonomously and preferentially regulates dendrite morphology. Interestingly, subcellular localizations of Golgi apparatus and mitochondria were also severely affected. These results indicate the requirement of intracellular functions of Vap33 in neural development. Moreover, to examine the roles of the secreted Vap33 MSP domain, we searched the important sequence required for Vap33 MSP secretion and succeeded to generate the “unsecretable *vap33* mutant” whose Vap33 MSP is not secreted but can function intracellularly. The unsecretable *vap33* mutant exhibited lethality. Overexpressing the unsecretable Vap33 in the *vap33*$^{-/-}$ PN clones rescued the dendritic defects, implying that the intracellular functions of unsecretable Vap33 are likely to be normal. From these results, we propose that secreted MSP domain plays important extracellular functions to maintain organismal viability. We’d like to discuss how secreted MSP domain contributes the animal physiology, which may lead the clues for the therapeutic strategy of neurodegenerative diseases.

991V Characterizing synaptic deficits at adult neuromuscular Junctions in a model of Amyotrophic Lateral Sclerosis *Jessica Sidisky*, Brandon Hocking, Nikki Huhulea, Sara Moran, Bali Connors, Daniel Babcock Lehigh

The hallmark of neurodegenerative diseases such as Alzheimer’s Disease (AD), Parkinson’s. Disease (PD), and Amyotrophic Lateral Sclerosis (ALS) is the loss of neurons. However, recent evidence suggests that synapse dysfunction occurs long before clinical symptoms are manifested. Although PD and AD are the most prevalent neurodegenerative diseases, ALS has the most rapid onset of symptoms from the time of clinical diagnosis. Although there has been significant progress made in understanding the disease, we still lack effective cures or treatments. Here we introduce an adult model of ALS to characterize the progression of synaptic deficits that precede motor neuron loss. Using a tissue specific-Gal4 and the temperature sensitive Gal80 to express human and mutant *fused in sarcoma* (*FUS*), a gene responsible for a genetic form of ALS, in the flight motor neurons in a spatial and temporally controlled manner. Synaptic deficits are characterized using the flight behavioral assay as a direct read out of flight motor neuron function. We found that overexpression of mutant FUS demonstrates a progressive loss of flight ability. We further define these synaptic defects using synaptic markers to understand how and when neuromuscular junctions (NMJs) are impaired before motor neuron loss. We also characterize the adult ventral abdominal NMJs in the same manner using a motor neuron driver specific to these NMJs. With this model, we hope to elucidate the mechanisms that underly synapse loss in a structural and functional manner.

992V Targeted downregulation of *Hipp1* ameliorates tau-engendered deficits in *Drosophila melanogaster* *SUNG YEON PARK*[1,3], JIEUN SEO[2], Seulbee Lee[2], Sang Jeong Kim[1,2,3], Yang-Sook Chun[1,2,3] 1) Ischemic/Hypoxic Disease Institute, Seoul National University, College of Medicine, Seoul, Republic of Korea; 2) Department of Biomedical Sciences, Seoul National University College of Medicine, Seoul, Republic of Korea; 3) Department of Physiology, Seoul National University

College of Medicine, Seoul, Republic of Korea

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-engendered eye defects, locomotion defects, reduced lifespan, weight loss, and neurodegeneration by preventing hyperphosphorylation of tau. 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.

993V De novo missense mutations in E3 ubiquitin ligase RNFT2 lead to intellectual disability as evidenced by loss of function studies in *Drosophila* *Ayşe Kahraman*[1, 2], Barış Can Mandacı[1, 2], Reza Ataei[1, 3], Kenneth Schöneck[1], Anastasia Fokina[1, 2], Çiğdem Soysal[1], Elif Darbuka[1, 2], Ibrahim Yaman[1, 2], Kimia Kahrizi[4], Hossein Najmabadi[4], Mohammad Haddadi[3], Arzu Çelik[1, 2] 1) Bogazici University, Department of Molecular Biology and Genetics, Bebek, Istanbul, Turkey; 2) Bogaziçi University, Life Sciences Center, Bebek, Istanbul, Turkey; 3) University of Zabol, Department of Biology, Faculty of Science, Zabol, Iran; 4) University of Social Welfare and Rehabilitation Sciences, Genetics Research Center, Tehran, Iran

Characterized by various deficiencies in both intellectual ability and adaptive behavior, intellectual disabilities (IDs) affect 1% of the population worldwide. Mutations in over 400 genes have been implicated in ID, however the underlying molecular links between genotype and phenotype for most of these genes remain unknown. Whole exome sequencing (WES) of a large cohort of 404 Iranian consanguineous families with autosomal recessive intellectual disability (ARID) led to the identification of a novel likely pathogenic missense variant (cT1150C; p.C384R) in the *RNFT2* gene. RNFT2 (RING finger transmembrane domain containing protein 2, also named as TMEM118), encodes a RING finger E3 ubiquitin ligase. Its fly ortholog *dmel/*CG13605 has the same type of RING domain (C3HC4 type) and is predicted to be a ubiquitin E3 ligase involved in the ER-regulated protein degradation pathway.
How disruption of RNFT2 gives rise to ID phenotypes is not known. To address this question, we sought to investigate the neurobehavioral consequences of losing the RNFT2 homolog in flies and identify its expression in the fly brain. In parallel, we investigated its subcellular localization in stably transfected HeLa cells. These cells will be used to identify the substrates of RNFT2.
Towards this end we have generated a Gal4 line and show that CG13605 is expressed in a subset of Kenyon cells in the fly brain starting at larval stages. Downregulation of CG13605 in the mushroom body with OK107-Gal4 lead to neurodevelopmental phenotypes such as loss of alpha and alpha' lobes. For a more detailed functional characterization, we generated null mutant flies using the CRISPR/Cas9 system. Loss of CG13605 led to phenotypes such as alpha lobe loss or beta lobe fusion. The observed defects in mushroom body development, which is the primary memory and learning center of flies, provide evidence for RNFT2 being an ID gene. Ongoing experiments including rescue experiments, analyses using patient-specific variants of RNFT2 as well as behavioral studies are expected to further support this hypothesis.

994V Homologues of the human disease-associated amyloidogenic proteins APP and TGFBI are required for physiological protein aggregation in *Drosophila* secondary cells *Clive Wilson*, Preman J Singh, Adam Wells, Claudia C Mendes, S Mark Wainwright, Pauline P Marie, Ben Kroeger, Deborah C I Goberdhan University of Oxford

Amyloidogenesis, the aggregation of soluble proteins into insoluble fibrils, has multiple biological functions in both health and disease. It is critically important in neurodegenerative disorders; for example, aggregations of A-beta peptides, cleaved products of Amyloid Precursor Protein (APP), form plaques in Alzheimer's Disease (AD). Mutated Transforming Growth Factor-Beta-Induced (TGFBI), an extracellular fibrillar protein, assembles into amyloid that leads to corneal dystrophies. Proteins also aggregate naturally, sometimes via amyloidogenesis, for example, when peptide hormones condense into insoluble, inert dense-core granules (DCGs), which are stored in secretory vesicles until release. Understanding the cell biology that drives these normal and pathological processes could reveal the key mechanisms that distinguish them, but visualising these events as they take place in cells and tissues is challenging. Here we describe the development of a new cellular model for DCG biogenesis, the *Drosophila* prostate-like secondary cell (SC). These cells have highly enlarged (5 micron diameter) DCG compartments, permitting DCG assembly to be followed by light and fluorescence microscopy in living tissue and in real-time. Remarkably, the rapid formation of DCGs requires the complementary activities of the fly homologues of TGFBI, called MFAS, and APP, called APPL. While DCGs cannot form

in the absence of MFAS, several smaller DCGs are often made in a single compartment in the absence of APPL, but they frequently cannot coalesce into a single large DCG, as happens in normal cells. Expressing a pathological, amyloidogenic form of human A-beta or TGFBI in SCs alters MFAS assembly in DCGs and can suppress DCG disassembly when secreted. Genetic manipulations that mirror changes seen in AD patients also modulate DCG biogenesis and disassembly. Interestingly, intraluminal vesicles (ILVs), which are secreted as exosomes, are formed in SC DCG compartments. We show they are also required for assembly of a large DCG, consistent with recent findings linking ILVs and exosomes to amyloidogenesis. Our studies, therefore, demonstrate that both APP and TGFBI have physiological, as well as pathological, modes of rapid protein aggregation, which can uniquely be distinguished by real-time fluorescence imaging in living SCs. We are employing this model to screen genetic modifiers of pathological versus physiological amyloidogenesis, which might highlight new ways to target amyloidogenic diseases.

995V Novel dominant and recessive variants in human *ROBO1* cause distinct neurodevelopmental defects through different mechanisms *Yan Huang*[1,2], Mengqi Ma[1,2], Xiao Mao[3,4], Davut Pehlivan[1,5,6], Oguz Kanca[1,2], Gulsen Akay[1], Tadahiro Mitani[1], Shenzhao Lu[1,2], Sukru Candan[7], Bo Xiao[8], James Lupski[1,6], Hugo Bellen[1,2] 1) Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, Texas, USA ; 2) Jan and Dan Duncan Neurological Research Institute, Texas Children's Hospital, Baylor College of Medicine, Houston, Texas, USA ; 3) National Health Commission Key Laboratory of Birth Defects Research, Prevention and Treatment, Hunan Provincial Maternal and Child Health Care Hospital, Changsha, Hunan, China; 4) Department of Medical Genetics, Maternal and Child Health Hospital of Hunan Province, Changsha, Hunan, China; 5) Division of Neurology and Developmental Neuroscience, Department of Pediatrics, Baylor College of Medicine, Houston, Texas, USA ; 6) Texas Children's Hospital, Houston, Texas, USA ; 7) Medical Genetics Section, Balikesir Ataturk Public Hospital, Balikesir, Turkey; 8) Neurology Department, Xiangya Hospital, Central South University, Changsha, Hunan, China

The Roundabout (Robo) receptors present on growth cones of neurons induce axon repulsion in response to the extracellular ligand Slit. The Robo family of proteins controls midline crossing of commissural neurons during development in vertebrate and invertebrate model organisms. Mono- and bi-allelic loss-of-function (LoF) of human *ROBO1* (MIM: 602430) has been associated with a breath of phenotypes, including neurodevelopmental defects such as strabismus, pituitary defects, intellectual impairment, as well as non-neuronal defects in heart and kidney, with reduced penetrance and highly variable expression. We identified two novel *ROBO1* variants associated with distinct phenotypes. In family #1, we identified a biallelic missense (*p.S1522L*) variant in three affected siblings with a recessive trait isolated nystagmus. In family #2, we identified a *de novo p.D422G* variant in the proband who presented with a severe early-onset epileptic encephalopathy. To assess the perturbance to biological homeostasis for these variants, we performed functional assays in *Drosophila*. We generated a null allele of *robo1* by inserting a CRIMIC T2A-GAL4 in an intron. To our surprise, transheterozygous *robo1* null mutation (*T2A-Gal4/Df*) leads to reduced viability but not lethality. In contrast, overexpression of either human *ROBO1* or fly *robo1* is toxic but also reduces viability. Note that human *ROBO1* is not able to replace fly *robo1* when driven by the *Gal4* insertion. The fly cDNA driven by the *Gal4* rescues the midline crossing at 18°C. However, the *p.D413G* variant in the fly cDNA fails to rescue midline crossing suggesting that it is a LoF allele. The recessive *ROBO1* variant cannot be tested as the *p.S1522* is not conserved in fly *robo1.*
We therefore turned to gain-of-function (GoF) assays using the *T2A-GAL4* to drive the human reference cDNA which leads to toxicity. *ROBO1 p.S1522L* is less toxic than the reference human cDNA with respect to viability and midline crossing. In contrast, the dominant *p.D413G* variant leads to a highly aberrant protein distribution of fly Robo1 and creates novel defects in several assays. This suggests that it is a neomorphic allele. In summary, our studies expand the phenotypic spectrum associated with *ROBO1* variant alleles and assesses the potential nature of the variants.

996V Localization of transgenes for *Drosophila* models of myotonic dystrophy type 1 *Andrea Waltrip*, Noah Smith, Ginny Morriss University of Mary Washington, Fredericksburg, VA

Myotonic Dystrophy Type 1, DM1, is a multi-systemic disorder that results from expansion of CTG repeats in the *DMPK* gene in humans. *Drosophila melanogaster* has been established as a model organism for the study DM1, by the construct of multiple transgenic DM1 lines containing different numbers of CTG repeats (60, 250, and 480), expressed using the GAL4/UAS system. Expression of long-repeat transgenes ($(CTG)_{250}$ and $i(CTG)_{480}$) has been shown to produce the phenotypes consistent with DM1, relative to control lines ($i(CTG)_{60}$). 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 in the genome. To narrow down location to a specific chromosome, genetic crosses using GAL4 drivers on different chromosomes are being used to drive expression of repeat expansions to assess phenotypic ratios of eye color traits, specific to the transgenes, and flight capability, which has been shown to be defective in DM1 flies. We expect to see different phenotypic ratios in the F_2 progeny from crosses using drivers localized to different chromosomes. We will confirm our chromosome localization using fluorescent in situ hybridization (FISH) of polytene chromosome preparations, using probes specific for the transgene insertion. Chromosome specific probes will be used to verify the chromosomal location. Since the FISH will allow us to narrow down the location of transgene insertion to a more specific

region of the chromosome, we can target that specific region to more precisely determine the insertion site using PCR and sequencing. Preliminary results from the genetic analysis suggest that the $(CTG)_{250}$ transgene is likely localized to chromosome 2 and that i$(CTG)_{480}$ and i$(CTG)_{60}$ are not likely localized to the chromosomes 1 or 2. Knowing the location of the transgenes can allow for more practical mating schemes to study various aspects of myotonic dystrophy disease mechanisms, but can also provide crucial information for understanding the expression of the transgenes, which may be influenced by nearby regulatory elements in the genome.

997V Increased oxidative stress precedes activation of the seizure-exacerbating glial immune response in *prickle* mutants *Krishna Madhav Nukala*[1], Anthony Lilienthal[1], Shu Hui Lye[2], Alexander Bassuk[3], Stanislava Chtarbanova[2], J. Robert Manak[1,3] 1) Department of Biology, University of Iowa, Iowa City, IA; 2) Department of Biological Sciences, University of Alabama, Tuscaloosa, AL; 3) Department of Pediatrics, University of Iowa Carver College of Medicine, Iowa City, IA

Epilepsy is a neurological disorder characterized by seizures and affects ~1% of the U.S. population. We have previously shown that mutations in *prickle* (*pk*) cause spontaneous myoclonic-like seizures and ataxia in *Drosophila*, similar to what is observed in humans carrying *PRICKLE* mutations. Moreover, a transcriptome analysis of *pk* mutant brains revealed a robust increase in glial-mediated innate innate immune response (GIIR), suggesting that the immune response might be connnected to seizure progression. Using genetic, behavioral and immunohistochemical methods, we also showed that inhibiting the GIIR leads to a reduction of neurodegeneration which in turn suppresses the age-related exacerbation of seizures in *pk* mutants. However, while these experiments were the first to genetically demonstrate a link between a brain-derived innate immune system activation and epilepsy exacerbation, the link between *pk* neuronal dysfunction and activation of the GIIR remained unclear. Several studies have linked increased oxidative stress to numerous neurological disease models including epilepsy, and we observe significant upregulation of antioxidant genes in our transcriptome data, suggesting that oxidative stress might be the initiating factor for GIIR and eventual seizure exacerbation. Here, we show that larval, but not adult, *pk* mutant brains show increased neuronal oxidative stress, while only adult *pk* mutant brains show a significant increase in antioxidant and innate immune responses. Together, these data show that oxidative stress precedes the activation of GIIR and may be responsible for generating the signal to the glia, thus identifying a potential mechanism for GIIR-associated seizure generation in *pk* mutants.This work is supported by NIH/NINDS R01NS098590.

998V Adding low levels of omega-3 and omega-6 fatty acids to the diet eliminates seizure-like activity and paralysis and alters gene expression in the bang-sensitive mutant *technical-knockout* Patrick Kuebler[1], Anelise Hutson[2], Masashi Tabuchi[2], *Daniel Kuebler*[1] 1) Franciscan University of Steubenville, Steubenville, OH; 2) Case Western Reserve University School of Medicine, Cleveland, OH

There is an increasing amount of evidence that dietary and metabolic alterations can help reduce seizure susceptibility. The seizure-susceptible Drosophila bang-sensitive (BS) mutants represent a good model system for investigating the link between diet, metabolism, and seizure activity. The BS mutants exhibit seizure-like activity (SLA) and paralysis following a variety of stimuli including mechanical vortexing. Previous work demonstrated that feeding the BS mutants standard media supplemented with a commercial ketogenic dietary mix (10% w/v) could eliminate the seizure-like activity and paralysis seen in these mutants. Given the mix of lipids in the supplement, this study examined the ability of specific polyunsaturated omega fats to alleviate the seizure-paralysis phenotype in one of the BS mutants, *technical knockout* (*tko*). The *tko* flies were fed 10% (w/v) sucrose-only media supplemented with one of two omega-3 fatty acids, eicosapentaenoic acid and docosahexaenoic acid, or one omega-6 fatty acid, arachidonic acid. The omega fatty acids were added at a final concentration of 25 µg/ml of media and 1-2 day old *tko* male flies were reared in the media for 3 days prior to testing for bang sensitivity. All three of the omega fatty acids eliminated the SLA and paralysis normally seen in *tko* flies following mechanical shock. Following the 3-day exposure to the omega fatty acids, the flies were then switched back to standard media for ten days and retested for bang-sensitivity. Following this period of feeding on normal media, the flies still did not exhibit SLA and more than 90% of the flies did not exhibit paralysis. This indicates the ability of the omega fatty acids to rescue the phenotype was maintained even when the flies were subsequently removed from the diet.
To investigate changes in gene expression associated with the feeding of omega fatty acids, RNA was extracted from the heads of *tko* flies fed 1) docosahexaenoic acid (25 µg/ml), 2) arachidonic acid (25 µg/ml), and 3) control sugar-only media. The Affymetrix GeneChip Drosophila Gene 1.0 ST Array was then used to perform whole transcriptome analysis of these fly heads. When compared to flies on the control diet, the data demonstrated that a number of ribosomal proteins were highly upregulated in fly heads following both omega fatty acid supplementation diets. Given that previous researchers have seen alterations in ribosomal protein levels in dendrites and axons following cellular changes, the increases seen here may be associated with synaptogenesis and neural alterations that modify excitability of the *tko* nervous system.

999V Kek1 inhibition of EGFR signaling: a Domain V mediated event *Joseph Duffy*, Alexander Putnam, Sarah Doherty Worcester Polytechnic Institute, Worcester, MA

The EGFR/ErbB family of receptor tyrosine kinases function in numerous developmental contexts and their dysregulation has been linked to various types of cancers. With receptor activation underlying their roles in cancer, molecules that inhibit EGFR/ErbB activity provide potential therapeutic avenues. One such inhibitory molecule is Kekkon1 (Kek1), a member of the *Drosophila* family of leucine-rich repeats (LRR) and immunoglobulin domain (Ig) containing transmembrane molecules. Kek1's extracellular LRRs, along with Domain V of the *Drosophila* receptor, are critical for binding. It has also been reported that Kek1 is able bind in a cross-species fashion the mammalian ErbB receptors. However, while the extracellular regions of both mammalian and *Drosophila* receptors contain Domains I-IV, mammalian receptors lack Domain V. Therefore, to clarify the interaction of Kek1 with the receptors and elucidate the role of Domain V, we sought to further define the mechanism of binding and inhibition. Using an ELISA-based assay, a battery of wild-type, chimeras, and variants of the *Drosophila* and human receptors were assessed for interactions with Kek1. Our results demonstrate that Kek1 binds the *Drosophila* and not the human receptors, and that specificity occurs principally through Domain V, which is absent in the human receptors. Our results also implicate differences between Domain IV in the *Drosophila* and human receptors in further restricting Kek1's interaction to the *Drosophila* receptor. Mechanistically, our results support a model in which Kek1 binds to Domain V on the dimerization and not the ligand-binding interface of the *Drosophila* receptor, thereby inhibiting receptor dimerization and consequent activation. Our work provides deeper insight to the structural requirements underlying Kek1's interaction with dEGFR and has clear implications for the proposed use of Kek1 as a therapeutic.

1000V RASopathy Drug Discovery Aimed at Treating Hypertrophic Cardiomyopathy *Kimberly Stephens*[1], Jared Gatto[1], Jianping Hu[1], Cèline Guichard[2], Rupa Mirmira[1], Tirtha Das[1], Husnu Kaniskan[1], Jian Jin[1], Ross Cagan[3], Bruce Gelb[1] 1) Mount Sinai School of Medicine; 2) Aix Marseille University; 3) University of Glasgow

Introduction: RASopathies are pleiomorphic genetic traits, predominantly resulting from gain of function in RAS/MAPK signaling. Hypertrophic cardiomyopathy (HCM) is a leading therapeutic target because of its association with early mortality in affected infants. MEK inhibitors have shown promise in pre-clinical studies and compassionate-usage in patients but are not curative, have dermatological side effects and are not uniformly efficacious. Developing RASopathy therapeutics that can be administered long-term while allowing homeostatic levels of RAS signaling is challenging. To address that, we used phenotype-driven, whole-organism chemical screening in Drosophila RASopathy models to drive therapeutic discovery.
Hypothesis: Using a fruit fly-based platform, we can identify novel small molecular therapeutics for RASopathies.
Methods: We generated transgenic Drosophila bearing pathogenic alleles associated with HCM and screened a chemical library for lead compounds based on rescuing lethality. We chemically evolved our best hit, M1, iteratively and assessed rescue efficacy. We assessed putative targets using similarity ensemble analysis (SEA).
Results: The parent M1 compound showed weak efficacy toward transgenic RAF1-S257L flies with 10 uM dosing in fly media. JH107-7 was the best M1 derivative for the RAF1 model, achieving 50% rescue with 1 uM dosing. Testing in other RASopathy fly models revealed nearly complete rescue in flies expressing PTPN11-N308D but not BRAF-W531C. M1 derivative JH93-178T rescued 45% of BRAF flies but was not effective for other fly models. To assess potential as cancer therapeutics, we tested our M1 derivatives against two RAS-driven colon cancer fly models for which no single drug has shown efficacy. For JH107-7 and JH93-178T, we nearly matched efficacy of combination chemotherapy with trametinib and zoledronic acid, a drug combination effective in flies and a patient. JH107-7's top 10 target predictions included RAF1, BRAF and mTOR.
Conclusion: Two M1-logs show efficacy for RASopathies and cancer in transgenic fly models with likely in-animal dosing of 5-50 nM (1/200 of concentration in food). Preliminary data with human RASopathy-mutant induced pluripotent stem cell-based HCM modeling suggest efficacy in rescuing cellular hypertrophy. We are currently assessing M1 derivatives' impact on signaling pathways.

1001V How polyploid cells become tumor and how fly deals with it? *Xian-Feng Wang*, Chun-Ming Lai, Chih-Hsuan Chang, Hongcun Bao, Deeptiman Chatterjee, Yi-Chun Huang, Wu-Min Deng Tulane University School of Medicine

Polyploid cells are frequently observed in human cancers and are positively correlated with malignancy and poor prognosis. Genomic instability and polyaneuploidy which caused by polyploid cells re-enter mitosis and depolyploidization have been well studied, the appropriate microenvironment by which polyploid cells re-enter mitosis has been elusive. Here, taking advantage of our polyploid *Drosophila* salivary gland imaginal ring tumor model which is driven by polyploid mitosis and depolyploidization and single cell RNA sequencing, we identified essential factors, including JNK, JAK/STAT and Hippo, which provide favorable soil for polyploid cells re-enter mitosis and become tumor. Besides tumor initiation factors, we found that tumor initiating polyploid cells show potential cell polarity defects which makes those cells easily lose cell polarity and become tumor. We further found the tumor initiating polyploid cells enrich cell death signal, such as *hid*, *rpr* and *Atg8*, leading to those ''dangerous cells'' being eliminated at metamorphosis during normal development. Escaping from cell death of those polyploid cells at the early development stages and tumor situation are largely depended on cell death inhibitor Diap1. Our findings reveal tumor microenvironments that provide a tumor formation soil for polyploid cell tumorigenesis.

1002V Towards understanding the mechanism of tumorigenesis caused by centrosome dysfunction *Chaitali Khan*, Nasser Rusan National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD 20892

Centrosome dysfunction is prevalent in many types of cancers; however, the mechanistic understanding by which centrosome defects cause cancer is still not very clear. Some studies have shown that centrosome amplification causes cancer by inducing mitotic errors and genomic instability. Other studies link centrosome dysfunction and tumorigenesis to the activation of a cellular stress response. Centrosome dysfunction can also lead to cancer by disrupting asymmetric stem cell division, which leads to errors in daughter cell fate. In *Drosophila*, genetic perturbations that disrupt *Drosophila* neural stem cells (NSC) asymmetric division, including centrosome mutations, give rise to tumors. Some of these mutations form tumors in the larva brain while others give rise to tumors upon transplanting NSC into the adult fly abdomen by an allograft method. We are using the allograft method to investigate tumorigenesis caused by compromised centrosome function in asymmetric stem cell division. Specifically, we are testing how does abnormal centrosomes affect the stem cell polarity and cell fate specification, genomic instability and how does this causes tumorigenesis of NSCs. Additionally, we are investigating the role of cellular stress response triggered due to dysfunctional centrsomes and its cooperation with tumorigenic signaling pathways in a cell autonomous or non autonomous manner. I will be discussing our findings addressing these questions.

1003V Using Drosophila Models to Dissect Biology and Signaling Mechanisms in Rare Drug Resistant Variants of Lung Cancer *Sereene Kurzum*[1], William Marsiglia[2], Chana Werther-Hecht[1], Bruce Gelb[1], Arvin Dar[2], Tirtha Das[1,3] 1) The Mindich Child Health and Development Institute, Dept. of Pediatrics, Department of Genetics and Genomic Science, Icahn School of Medicine at Mount Sinai; 2) Department of Oncological Sciences, Icahn School of Medicine at Mount Sinai; 3) Dept. of Cell, Developmental, and Regenerative Biology, Icahn School of Medicine at Mount Sinai, New York NY USA

Animal models and clinical data have vastly improved our understanding of gene variants that lead to human disease. In low occurrence cancers, rare gene variants arise in tumors of patients receiving targeted therapy. We had previously developed a Drosophila model for a rare lung cancer arising from a fusion oncogene KIF5B-RET and showed that this fusion protein assembles a complex signaling hub composed of multiple receptor tyrosine kinases (RTK's). Our finding predicted that drugs designed to inhibit the RET-kinase activity alone may be insufficient to fully suppress oncogenic signaling from this hub. Subsequently, multiple clinical trials have shown poor response of RET-inhibitor drug treatment in patients harboring KIF5B-RET fusions in their lung tumors. Recently a few drugs, like loxo-292, designed to specifically and potently inhibit RET-kinase have been developed. In our fly model, treatment of KIF5B-RET with loxo-292 prevented phosphorylation of RET and some components of the hub like EGFR, but not other components like FGFR. This result indicates a potential mechanism of how drug resistant compensatory pathways could arise even after treatment with RET-kinase selective inhibitors. Moreover, recent clinical studies have shown that KIF5B-RET positive lung cancer patients develop rare KIF5B-RET-variants while undergoing treatment with RET-kinase selective drugs, and that these drug resistant variants reduce efficacy of the treatment. We developed one such model, using the human KIF5B-RETG810R, and found that this variant enhanced RET-kinase domain phosphorylation and enhanced activation of various RTK dependent pathways of the KIF5B-RET hub. Indeed, none of the studied RTK pathways were suppressed when KIF5B-RETG810R fly tissues were treated with loxo-292. In summary, our current findings using Drosophila models indicate that RET-selective drugs like loxo-292 may not fully suppress signaling of KIF5B-RET or KIF5B-RETG810R variant. Our ongoing studies predict potential molecular mechanisms of drug resistance to RET-selective inhibitors in various cancers and highlight the value of combining whole animal Drosophila models with chemical genetics to study human disease.

1004V Rbf/E2F1-mediated transition from steroid-dependent to steroid-independent ecdysone receptor signalling in *Drosophila* prostate-like secondary cells *Aashika Sekar*[1,2], Mark Wainwright[2], Aaron Leiblich[2], Claudia Mendes[2], Clive Wilson[2] 1) Centre for Tumour Biology, Barts Cancer Institute, London, United Kingdom; 2) Department of Physiology, Anatomy and Genetics, University of Oxford, United Kingdom

Castration-resistant prostate cancer (CRPC) is an incurable, androgen-independent form of prostate cancer that emerges in the hormone-depleted environment of androgen deprivation therapy (ADT). However, CRPC growth still frequently depends on Androgen Receptor (AR) signalling. Unusually, loss of the tumour suppressor gene, *Retinoblastoma* (Rb), and consequent activation of transcription factor E2F1 have been linked to late-stage tumour progression to CRPC, rather than early-stage events. We have previously shown that binucleate secondary cells (SCs) of the *Drosophila melanogaster* male accessory gland (AG) share several functional and signalling similarities with human prostate epithelial cells. Upon mating, SC growth regulation switches from a steroid-dependent to a steroid-independent form of Ecdysone Receptor (EcR) control that induces genome endoreplication. Here, we demonstrate that the *Drosophila* Rb homologue, Rbf, and E2F1, as well as cell cycle regulators, Cyclin D (CycD) and Cyclin E (CycE), are key mediators of SC growth and endoreplication both in virgin and mated males. Importantly, we show that the CycD/Rbf/E2F1 axis requires the EcR, but not ecdysone, to trigger CycE-dependent endoreplication and associated growth in SCs after mating, mirroring changes in CRPC. We also demonstrate that excess Rbf activity reversibly suppresses binucleation in adult SCs. Overall, our work reveals mechanistic parallels between the physiological switch to hormone-independent EcR signalling in SCs and the pathological switch seen in CRPC, and suggests that the latter may represent the dysregulation of a

currently unidentified physiological process, which permits AR signalling when androgen levels are low.

1005V Tissue specific knockdown of Scribble induces tumor progression and metastasis in *Drosophila* *Jyotsna Singh*, Saripella Srikrishna Department of Biochemistry,Institute of Science,Banaras Hindu University, Varanasi, UP 221005 ,India

Cancer is one of the deadly diseases worldwide and the cancer-related deaths across the globe will rise to over 11 million by 2030 and 27 million by 2050. Metastasis is the major cause of cancer associated mortality in humans. Metastasis is the process by which an uncontrolled malignant growth of tumor cell leaves the primary tumor and invades into distant regions from its site of origin, leading to formation of secondary tumors. Epithelial cell polarity regulator gene, *Scribble,* implicated in variety of cancers, plays major role in cancer progression and metastasis. *Drosophila* pupa is a valuable metastasis model for studying live dynamics of tumor micro environment and metastasis events by combing endogenous fluorescence probes and fluorescence microscopy. Here, we have generated a metastatic cancer in *Drosophila* by knockdown of *scribble* in wing imaginal discs using UAS-GAL4 system. We have found that *scribble* knockdown induced overgrowth of wing imaginal tissue leading to metastatic secondary tumor formation and absolute lethality at early pupal stage. Tumor progression and metastasis phenotypes in *Drosophila* were scored for 84 hours after puparium formation (APF) and recorded the invasion of GFP labeled tumor cells. Tumor bearing pupae showed spreading of secondary tumors cells throughout from anterior to posterior regions of the pupae while wild type pupae showed GFP expression restricted to developing wing discs areas only. Further, we checked mRNA expression of metastatic biomarkers at different time point of pupal development. The up-regulation of MMPs and reduced expression of *E-cadherin* were confirmed the increased cell proliferation and metastasis in *Scrib* knockdown tumorous tissues of pupae. Further study is in progress in this direction.
Keywords: *Drosophila*, Cancer, tumor, *scribble*, Metastasis

1006V Septins regulate heart contractility through modulation of cardiomyocyte store-operated Ca^{2+} entry *Benjamin Tripoli*, Courtney Petersen, Jeremy Smyth USUHS - Bethesda, MD

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 the pathway in which endoplasmic/sarcoplasmic reticulum (ER/SR) calcium store depletion causes influx of calcium to replenish the stores. The pathway is mediated by STIM proteins, which act as calcium sensors in the SR/ER, and ORAI calcium influx channels in the plasma membrane. Both upregulation and suppression of SOCE in cardiomyocytes causes disruption of normal cardiac function, demonstrating that proper regulation of SOCE in cardiomyocytes is essential. However, the mechanisms responsible for the regulation of SOCE in cardiomyocytes is not yet fully understood. To this end, septin GTPases have emerged as regulators of the SOCE pathway in non-cardiac tissues, with septin 1, 2, or 4 suppression resulting in SOCE suppression and septin 7 (PNUT) 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. We further show that co-expression of septin 2 RNAi with a constitutively active Orai channel suppresses the septin 2 phenotype, supporting the hypothesis that septin 2 associated dilated cardiomyopathy is due to SOCE suppression. 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 suppressed by Orai depletion, again supporting a role for SOCE dysregulation in the septin depletion-mediated cardiac phenotypes. We are currently developing tools to analyze Stim and Orai localization, sarcomere organization, and t-tubule architecture to further investigate septins and SOCE regulation in cardiomyocytes.

1007V Focus on the foci: Investigating the role of HDAC4 aggregation in neuronal development in *Drosophila melanogaster* *Hannah Hawley*, Helen Fitzsimons School of Natural Sciences, Massey University, New Zealand

Dysregulation of histone deacetylase 4 (HDAC4) expression and subcellular distribution has been observed in a number of neurodevelopmental and neurodegenerative diseases, and in our *Drosophila melanogaster* model, *HDAC4* overexpression impairs neuronal development and long-term memory. Interestingly, this is associated with minimal transcriptional changes. Upon increased abundance in nuclei, we observe HDAC4 aggregation into punctate foci, and therefore hypothesise that neuronal dysfunction mediated by HDAC4 overexpression is a result of aggregate formation.

The glutamine-rich N-terminus of HDAC4 forms an alpha helix which assembles into an unstable tetramer. To investigate whether HDAC4 aggregates contribute to neurodevelopmental deficits, transgenic *Drosophila* were generated which express HDAC4 mutants harbouring structure-guided substitutions of key amino acids important in mediating tetramerization. Expression of these mutant HDAC4 constructs significantly reduced aggregate formation in neuronal nuclei (ANOVA, $p < 0.01$), and this correlated with a significant reduction in defects in axon morphogenesis (Fisher's

exact, $p < 0.01$) and photoreceptor development (ANOVA, $p < 0.01$), as compared to wild-type HDAC4. These data suggest HDAC4 aggregation is at least in part responsible for neurodevelopmental and neurodegenerative disease in which HDAC4 is aberrantly expressed, and warrants further studies into the composition of these aggregates as well as strategies to mitigate their formation.

1008V De novo variants in SUPT16H are associated with developmental delay, intellectual disability, epilepsy and facial dysmorphism *Mengqi Ma*[1,2], Xiao Mao[3,4], Yiming Zheng[1,2], Shenzhao Lu[1,2] 1) Baylor College of Medicine, Houston, TX; 2) Jan and Dan Duncan Neurological Research Institute, Texas Children›s Hospital, Houston, TX; 3) National Health Commission Key Laboratory of Birth Defects Research, Prevention and Treatment, Hunan Provincial Maternal and Child Health Care Hospital, Hunan, China; 4) Department of Medical Genetics, Maternal and Child Health Hospital of Hunan Province, Hunan, China

We identified two individuals from two families carrying de novo missense variants in SUPT16H. The patients exhibit global developmental delay, intellectual disability, epilepsy, facial dysmorphism and structural abnormalities of the brain. SUPT16H is conserved across species and encodes the large subunit of the FACT complex which functions as a nucleosome organizer during transcription. Bioinformatic predications from population genomics databases indicate that SUPT16H is highly constrained for loss-of-function variants in humans and both missense variants p.T171I and p.G808R have not been reported to date. We employed *Drosophila* to characterize these two variants. We show that loss of the fly orthologue, *dre4,* causes early lethality. Tissue-specific RNAi-mediated knockdown of *dre4* in the eye or wing disc leads to loss of eye and wing, respectively. RNAi-mediated knockdown in the nervous system also causes severe defects. Expression of the human reference *SUPT16H* transgene partially rescued the loss-of-function phenotypes in wing, eye and nervous system. However, the p.T171I or p.G808R variants are much less efficient at rescuing these phenotypes, indicating that they are partial loss-of-function alleles. We show that *dre4* functions in a cell-autonomous manner, rather than in an endocrine fashion dependent on ecdysteroid production as previously proposed. Altogether, our data indicate that de novo loss-of-function variants in SUPT16H are associated with neurological features.

1009V Drosophila models reveal nuclear shape and lamin localization patterns that differentiate clinically distinct laminopathies Sydney Walker[1], Laura Hecker[2], *Lori Wallrath*[1] 1) University of Iowa; 2) Clarke University

Laminopathies are a collection of diseases caused by mutations in the human *LMNA* gene, which include muscular dystrophy and lipodystrophy, an adipose tissue disorder. The symptoms and severity of laminopathies vary depending on the genetic background of the individual. The *LMNA* gene encodes lamins A/C, intermedicate filaments that form a meshwork underlying the inner nuclear membrane. Generating Drosophila models allows for analysis of specific mutations in a defined genetic background and the ability to express mutant lamins in specific tissues. Thus, we modeled two *LMNA* mutations that cause distinct clinical symptoms in *Lamin C*, the Drosophila orthologue of human *LMNA*. Both mutations cause a single amino acid substitution in the conserved Ig-like fold domain of lamins. Lamin A/C Arg482Trp causes lipodystrophy; lamin A/C Arg527Pro causes Emery-Dreifuss muscular dystrophy and lipodystrophy. Using the Gal4/UAS system, wild-type and mutant lamins were expressed in larval body wall muscles and cardiac tissue. When expressed in the larval body wall muscles, lamin Arg527Pro aggregated within the cytoplasm and caused reduced larval motility. By contrast, lamin Arg482Trp caused abnormally shaped nuclei and had no effect on larval motility. Surprisingly, both mutants caused death at the pupal stage. When expressed in cardiac tissue, lamin Arg527Pro caused a shortened adult lifespan. By contrast, flies with cardiac-specific expression of lamin Arg482Trp exhibited a lifespan similar to that of flies expressing wild-type lamin. Thus, the Drosophila models recapitulated aspects of the human disease, with only Arg527Pro affecting muscle function. In addition, the distinct intracellular localization patterns and differing effects on nuclear morphology provide an explanation for how mutations that affect the same lamin domain cause distinct clinical pathology.

1010V Generating *Drosophila melanogaster* isofemale lines tolerating extreme oxygen conditions *Dan Zhou*[1], Jin Xue[1], Tsering Stobdan[1], Gabriel Haddad[1,2,3] 1) Department of Pediatrics, University of California San Diego; 2) Department of Neuroscience, University of California San Diego; 3) The Rady Children›s Hospital

Hypoxic and oxidative stress are common pathological elements in many diseases. In order to understand the mechanisms underlying mechanisms regulating tolerance or susceptibility to hypoxic or oxidative stress-induced injuries, we developed two *Drosophila melanogaster* populations that can live perpetually in severe, normally lethal, hypoxic or hyperoxic environments through experimental evolution. From these populations, we generated a Panel of Low Oxygen Tolerant (PLOT) and a Panel of High Oxygen Tolerant (PHOT) isofemale lines (total 79 PLOT and 65 PHOT lines) to identify and study specific genetic mechanisms that are responsible for the hypoxia, or hyperoxia, tolerant trait. These isofemale lines showed significantly enhanced hypoxia or hyperoxia tolerance as compared to their wildtype parental and the wildtype DGRP isofemale lines. For example, 95% of the PLOT lines showed hypoxia tolerance with eclosion rate >50% at 5% O_2 level. In contrast, none of the parental lines and only 15% of the DGRP lines had similar eclosion rates under the same hypoxic condition. Furthermore, under the lethal condition with 4% O_2, the PLOT lines exhibited a wide range of eclosion rate from 1% to 80% with a clear pattern of Gaussian-Distribution. These PLOT and PHOT flies may provide

us a unique opportunity to study the molecular basis of stress-directed evolution, such as the role of genetic variations in the protein coding regions and functional DNA elements in hypoxia, or hyperoxia, adaptation. In addition, these PLOT and PHOT lines can also be used to study phenotypic plasticity as well as genetic-epigenetic interactions in hypoxic or oxidative environments.

1011V Effect of Circadian Rhythm Disruption on DNA Double Strand Break Repair Pathway Choice Lydia Bergerson, Caleb Fitzmaurice, Tyler Knudtson, Halle McCormick, *Alder Yu* University of Wisconsin - La Crosse

Long-term night shift work has been associated with increased risk of certain cancers, although this link is not consistently supported. The most obvious difference between shift workers and non shift workers is increased disruption of circadian rhythms in shift workers. If circadian rhythm disruption increases use of mutagenic DNA double strand break repair (DSBR) pathways such as non-homologous end joining (NHEJ), that would provide a potential mechanistic link between shift work and cancer initiation.
To test the hypothesis that circadian rhythm disruption increases use of NHEJ DSBR, we used the Rr3 system. The Rr3 system is based on a chromosomally integrated DsRed gene that is rendered nonfunctional by an inserted I-*Sce*I site flanked by a short direct repeat. Rr3 containing flies are crossed to I-*Sce*I expressing flies and DSBs are induced in the F1 generation premeiotic germline. Individual repair events are recovered and scored in the F2 generation by appropriate crosses. Repair by base pairing at the direct repeats (single strand annealing, SSA) reconstitutes expression of the DsRed gene. Repair by NHEJ does not reconstitute DsRed expression. We compared relative usage of SSA and NHEJ repair pathways between flies maintained on 12:12 and 8:8 light:dark schedules. Disruption of circadian rhythms by the 8:8 schedule was confirmed by actimetric analysis. We found no significant difference in NHEJ or SSA repair pathway usage between 12:12 and 8:8 flies. Current experiments are investigating the effect of circadian rhythm disruption on use of homologous recombination repair.

1012V Elucidation of the role of *IFT52* associated with a novel skeletal ciliopathy using *in vitro* and *Drosophila* systems *Vishal Singh Guleria*, Rahul Parit, Girisha M Katta, Priyanka Upadhyai Kasturba Medical College, Manipal, India

Primary cilia (PC) are tubular antenna like structures present on the mammalian cell surface transducing extracellular cues like hormones, growth factors and mechanical stimuli. Also, playing an important role in cellular development, differentiation and homeostasis. These microtubular structures are maintained by intraflagellar transport (IFT) machinery involved in bidirectional transport of ciliary proteins. *IFT52* is a component of the IFT complex and associated with a novel skeletal ciliopathy, short-rib thoracic dysplasia 16 with or without polydactyly (MIM# 617102). Cellular mechanism of *IFT52* function in modulating primary cilia biogenesis, signalling and its dependent regulation of osteogenesis and chondrogenesis is not known.
In our study we have characterized PC during osteogenic (OS) and chrondrogenic (CH) differentiation *in vitro* and elucidated the molecular mechanism of *IFT52* function in a PC dependent manner during osteogenesis and chondrogenesis in C3H10T1/2 and ATDC5 cell lines and *Drosophila* systems. OS and CH differentiation was induced in both cell lines and ascertained by assaying for known differentiation markers, calcium and glycosaminoglycan deposition in the extracellular matrix. *Ift52* transcript levels were studied using qRT-PCR. Additionally, we characterized the *IFT52* ortholog, *Osm6* in *Drosophila* by *IFT52* knockdown (KD) in embryonic neuroblasts by RNA interference using pan neuronal *Gal4* drivers.
OS differentiation was evident at 7 days following induction via alizarin red (AR) staining to reveal extracellular matrix (ECM) mineralization. CH differentiation was first detected at 14 days post induction by sulfated proteoglycan deposition and ECM mineralization using alcian blue and AR staining. We found *Ift52* transcript levels were upregulated at 14 and 21 days of OS differentiation, while in CH differentiation they were initially upregulated at 7 days and subsequently depressed. *Ift52* KD resulted in reduced protein expression and significant reduction of PC prevalence, length and OS differentiation.
IFT52 KD in embryonic neuroblasts of *Drosophila melanogaster* resulted in significant loss of chordotonal function like locomotion and hearing in adults and larva. Also, *IFT52* KD embryos showed structural ciliary defects like profound depletion of the Ich5 cilia in embryos. Our findings are anticipated to remit a comprehensive molecular characterization of the *Ift52* gene and enhance our understanding of its allied human anomalies.

1013V Genetic investigation of the Endolysosomal Network in a *Drosophila* model of Alzheimer's disease *Sher Li Tan*[1,2], Robert Richards[1], Tim Sargeant[2], Louise O'Keefe[1,2] 1) The University of Adelaide, Adelaide, South Australia, Australia; 2) South Australian Health and Medical Research Institute, Adelaide, South Australia, Australia

Introduction: Alzheimer's disease (AD) is the most common form of dementia, which affects memory, behaviour and daily functioning of affected patients with its pathological characteristics consisting of amyloid-beta (Aβ) plaques and phosphorylated tau accumulation in the brain. The mechanisms underlying the disease remain unknown and no effective treatments are available to prevent disease progression. Although the genetic contribution in AD patients is not fully understood, genome-wide association studies (GWAS) have identified several loci associated with increased AD risk

in genes within the endolysosomal network (ELN). However, the fundamental mechanism of AD disease progression regarding ELN is not well defined. Therefore, this project aims to identify and characterise the contribution of individual ELN genes to neuronal dysfunction in a *Drosophila* model for AD.

Method/Approach: Co-expression of full length amyloid precursor protein and beta-site APP cleaving enzyme (APP+BACE) has been investigated in *Drosophila* to determine whether flies are able to model symptoms observed in AD patients. The advantage of this APP+BACE model is that it will ensure correct spatial localisation of APP and its proteolytic fragments within the ELN. Phenotypes generated form the basis for genetic modification analyses of individual ELN genes.

Result: Our findings show that co-expression of APP+BACE causes retinal degeneration in the adult eye and this can be modified by altered expression of Rab5 or Rab7 ELN genes. In addition, neuronal expression of APP+BACE in flies has led to progressive loss of motor functions, behavioural problems, and a reduced lifespan.

Conclusion: Using this system, we can characterise the genetic and molecular contribution of individual ELN genes to APP+BACE toxicity.

1014V Drosophila models of *SNRNP200*-retinitis pigmentosa exhibit retinal apoptosis and loss of photoreceptor function *Sara Mayer*, Quinton Christensen, Arlene Drack, Lori Wallrath University of Iowa

Retinitis pigmentosa (RP) represents a collection of genetic eye disorders that cause gradual loss of photoreceptors. The initial phases of RP involve loss of rod photoreceptor function, which leads to loss of cone photoreceptor function and eventual blindness. RP affects 1:4,000 people worldwide and is either syndromic or non-syndromic. RP33 is a non-syndromic form of RP caused by mutations in the *SNRNP200* gene, which encodes a core component of the spliceosome. SNRNP200 possesses helicase activity that plays a critical role in pre-mRNA splicing. SNRNP200 is expressed in nearly every cell type, yet defects are only observed in the retina. To understand the function of *SNRNP200* in vision, we turned to Drosophila. The Drosophila orthologue of human *SNRNP200* is *lethal(3)72Ab (*or *Brr2),* which we refer to as *dSNRNP200*. Human SNRNP200 and fly dSNRNP200 have 74% amino acid identity and 89% similarity. Null mutations in *dSNRNP200* are homozygous lethal. RNAi knock-down of *dSNRNP200* in the developing eye leads to increased apoptosis in the larval eye/antennal imaginal discs and an adult rough eye phenotype. To understand human disease mechanisms, we generated patient based CRISPR mutant alleles encoding single amino acid substitutions in the first helicase domain. The CRISPR alleles showed no obvious physical defects; however, upon investigation they had increased apoptosis in larval eye/antennal imaginal discs relative to controls. Furthermore, electroretinograms performed on young adults bearing the CRISPR mutant alleles exhibited abnormal waveforms indicative of loss of phototransduction and synaptic transmission that progressed with age, similar to the human disease condition. In addition, loss of prolonged action potential indicated that the “rod-like” cells of the retina, were defective. By contrast, the cone-like cells, appeared functional, recapitulating early stages of the human disease. Collectively, we found that globally expressed *dSNRNP200* is essential for Drosophila photoreceptor function and modeling RP33 mutations in the fly recapitulates many aspects of the human disease, allowing for a molecular genetic dissection of disease mechanisms.

1015V Mutagenic-Antimutagenic Effect from the extract of a Medical Plant: the Wormwood in the Drosophila SMART assay *Ana Cecilia Luis Casta?eda*[1], Dulce María de Jesús Bolaños Neria[2], Marco Antonio Carballo Ontiveros[3], América Nitxin Castañeda Sortibrán[4] 1) Universidad Nacional Autónoma de México; 2) Universidad Nacional Autónoma de México; 3) Universidad Nacional Autónoma de México; 4) Universidad Nacional Autónoma de México

In Mexico, around 4000 flowering plants are known for medicinal use, of which only 5% have previous studies on their chemical composition, pharmacology, safety, efficacy, and possible adverse effects. Wormwood (Artemisia absinthium) is a plant widely used in traditional Mexican medicine to remedy stomach pain, bile problems, antipyretic, and other conditions. The plant’s parts used for this purpose are its cooked leaves and branches, which are drunk on an empty stomach. However, there are no parameters that allow establishing an adequate dose according to its toxicity in the body. In this sense, the objective of this work is to know the possible mutagenic and antimutagenic effect of the aqueous extract of Artemisia absinthium L. through the somatic mutation and recombination test (SMART) in Drosophila melanogaster wing cells. The SMART assay is based on detecting the loss of heterozygosity, which can be the result of different events, such as mitotic recombination, point mutation, deletion, and nondisjunction, while using recessive markers that are expressed on the surface of the wings of flies, such as spots (clones) with multiple wing trichome (mwh) and/or flame-shaped (flr3) phenotype, rather than a single trichome per cell. The SMART assay commonly uses 2 crosses, the standard cross (ST) and the high bioactivation cross (BE). BE is characterized by a high constitutive level of cytochromes P450, facilitating the detection of promutagens and procancer. In this study, three concentrations (1, 5, and 10%) of wormwood extract were used in order to detect the possible mutagenic effect, as well as a combination of 4NQO (mutagenic compound) with 10% of wormwood to evaluate its possible antimutagenic effect. The data obtained allow us to conclude that wormwood does not have a mutagenic effect at any of the concentrations used. However, it was found

that it potentiates the mutagenic effect of 4NQO. In addition, it was observed that it does not have an antimutagenic effect either.

1016A Using expansion microscopy to examine parasegmental boundaries at nanoscale resolution *Samia Parveen*, Ian Millerschultz, Adam Pare University of Arkansas

Over the course of animal development, distinct pools of epithelial cells are maintained by compartment boundaries. For example, in the early *Drosophila* embryo, the formation of compartment boundaries at regular intervals along the anterior-posterior axis establishes the segmental body plan of the future animal. These so called "parasegmental boundaries" (**PSBs**) are composed of highly stable cell-cell contacts that are under increased tension compared with typical contacts, and they resist the movement of cells between adjacent compartments. However, it is still unclear what differentiates PSB contacts from normal contacts at a molecular level. The force-generating cytoskeletal proteins F-actin and myosin are more highly enriched at PSBs compared with normal contacts, and evidence suggests that E-cadherin-based adherens junctions may also be differently arranged at PSBs. However, the fine details of these structures are below the resolution limit of standard confocal microscopy. To get past this resolution limit without the need for expensive super-resolution microscopes, we have adapted existing expansion microscopy (**ExM**) protocols for use in the early *Drosophila* embryo. ExM involves the isotropic physical expansion of a sample through chemical treatments to swell the embryo in a controlled manner, maintaining the relative positions of all molecules. We are using this technique to examine the ultrastructural distribution of adherens junction and cytoskeletal proteins at PSBs to see how they differ from normal contacts. We are also examining two known upstream regulators of PSB formation, Tartan and Ten-m, to better characterize their subcellular localization patterns in the early embryo. We expect these studies will give us a better understanding of how the three-dimensional arrangement of the cellular effectors of tension and adhesion—as well as the cell surface proteins that regulate them—gives PSBs their unique properties.

1017B Adaptation of the CRISPR-Sirius tool for imaging the genome in Drosophila ovaries *Erica Berent*, Haosheng Li, Oscar Bautista, Joseph Terry, Bowen Man, Nicole Crown Case Western Reserve University

Traditional fluorescence in situ hybridization (FISH) techniques are destructive to chromatin structure, and they are labor intensive when combined with immunofluorescence. In order to label and image specific loci without relying on FISH, we have adapted the genetically encoded CRISPR-Sirius system (Ma et al 2018) for use in the Drosophila ovary. This system consists of three components: a nuclease dead Cas9, a modified guide RNA containing MS2 stemloops, and an MCP-fluorescent protein fusion that binds the MS2 stemloops. To adapt this system into flies, we first used site directed mutagenesis to mutate the catalytic sites of Cas9 in the nanos::Cas9 vector (Port et al 2014). Second, we cloned eight copies of the MS2 step loop (Ma et al 2018) into the core guide RNA in pCFD3 (Port et al 2014) to generate pCFD3-Sirius. Lastly, we used an existing nanos::MCP-GFP fusion protein stock (BDSC#63821). To test our system, we cloned a guide RNA targeting the 359 bp repeat into our new pCFD3-Sirius vector. We then crossed the pCFD3-Sirius-359 repeat flies to nanos::GFP-MCP; nanos::dCas9 flies and immunostained the ovaries with an antibody against GFP. We will present data showing that our system is able to target repetitive sequences in the Drosophila genome and we will present our progress in targeting unique non-repetitive sequences.

1018C An improved organ explant culture method reveals stem cell lineage dynamics in the adult *Drosophila* intestine *Marco Marchetti*, Chenge Zhang, Bruce Edgar Huntsman Cancer Institute, University of Utah, Salt Lake City, UT

In recent years, live-imaging techniques have been developed for the adult midgut of *Drosophila melanogaster* that allow temporal characterization of key processes involved in stem cell and tissue homeostasis. However, current organ culture techniques are limited to imaging sessions of <16 hours, an interval too short to track dynamic processes such as damage responses and regeneration, which can unfold over several days. Therefore, we developed a new organ explant culture protocol capable of sustaining midguts *ex vivo* for up to 3 days. This was made possible by the formulation of a culture medium specifically designed for adult *Drosophila* tissues with an increased Na^+/K^+ ratio and trehalose concentration, and by placing midguts at an air-liquid interface for enhanced oxygenation. We show that midgut progenitor cells can respond to gut epithelium damage *ex vivo*, proliferating and differentiating to replace lost cells, but are quiescent in healthy intestines. Using *ex vivo* gene induction to promote stem cell proliferation, we demonstrate that intestinal stem lineages can be traced through multiple cell divisions using live imaging. Both asymmetric and symmetric divisions can be identified in the reconstructed lineages. We find that daughter cells of asymmetric divisions remain in close proximity of each other, while the progeny of symmetric divisions actively move apart, with implications for cell differentiation and tissue organization. We show that the same culture set-up is useful for imaging adult renal tubules and ovaries for up to 72 hours. By enabling both long-term imaging and real-time *ex vivo* gene manipulation, our simple culture protocol provides a powerful tool for studies of epithelial biology and cell lineage behavior.

1019A Graphene Enabled Optical Cardiac Control of Drosophila *Abby Matt*[1], Hongwu Liang[1], Matthew Fishman[1], Andrey Komarov[1], Xinyuan Zhang[1], Jing Men[1], Elena Gracheva[1], Alex Savtchenko[2], Chao Zhou[1] 1) Washington University in

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Current methods of cardiac stimulation, such as electrical stimulation, ultrasound stimulation, and cardiac optogenetics have certain risks or drawbacks. The use of invasive surgical procedures to implant electrodes and electrochemical reactions caused by electrode-tissue contact may cause serious detrimental effects. In this study, we implement a stimulation technique in the fruit fly Drosophila melanogaster heart that uses graphene injection to optically modulate cardiac activity. Graphene is a single layer arrangement of carbon molecules with unique electrical properties. We prepare a homogenized graphene solution for injection into the heart tube with a combination of phosphate buffer saline (PBS) or artificial hemolymph (AH). PBS and AH were also injected as control solutions. From the graphene solution, optical stimulation excites graphene particles that coat the heart tube, which in turn creates electrical stimulation, changing the membrane potential of heart tube muscles. Through the use of an Optical Coherence Microscopy (OCM) system, custom red light simulation, and a machine learning based model for analysis, we demonstrate non-contact cardiac stimulation after injecting graphene-based biointerfaces into the heart. Further, the analysis of heart mechanics, such as fractional shortening, indicates that there is no change to the way the heart moves when stimulated. This system is promising for studies of various gene-related cardiovascular diseases or for testing drugs. For example, it could enable studies of various cardiovascular differences in Drosophila caused by human ortholog gene mutations.

1020B An expanded toolkit for gene tagging using synthesized homology donor constructs for CRISPR mediated homologous recombination *Oguz Kanca*[1,2], Jonathan Zirin[3], Yanhui Hu[3], Burak Tepe[1,2], Debdeep Dutta[1,2], Wen-Wen Lin[1,2], Liwen Ma[1,2], Ming Ge[1,2], Zhongyuan Zuo[1,2], Lu-Ping Liu[3], Robert W. Levis[4], Norbert Perrimon[3,6], Hugo J. Bellen[1,5] 1) Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX ; 2) Duncan Neurological Research Institute, Texas Children Hospital, Houston, TX; 3) Department of Genetics Harvard Medical School, Boston, MA ; 4) Department of Embryology, Carnegie Institution for Science, Baltimore, MD ; 5) Department of Neuroscience, Baylor College of Medicine, Houston, TX ; 6) Howard Hughes Medical Institute, Harvard Medical School, Boston, MA

Gene traps and protein traps are valuable genetic tools to study gene function. The majority of these constructs are inserted in the genome as artificial exons, with a Splice Acceptor (SA)-effector-Splice Donor (SD) configuration. The artificial exon must be inserted in an intron between two coding exons to function as a gene or protein trap. About 50% of the Drosophila genes do not have a suitable coding intron. We developed methods to replace the coding region of these genes with a Kozak-GAL4-3XP3GFP cassette creating a null allele, while expressing GAL4. We have generated >100 KozakGAL4 alleles to date. Analysis of the expression patterns in 3rd instar larval brains shows that ~80% of the tested KozakGAL4 alleles can drive expression of UAS reporter genes with discernible patterns. We selected KozakGAL4 alleles that drive reporter expression in restricted patterns and used publicly available single cell RNA sequencing (scRNAseq) data to find other genes that are co-expressed with the targeted gene. T2AGAL4 alleles of such genes, identified by scRNAseq, drive expression of reporter genes in patterns overlapping with the KozakGAL4 targeted genes. scRNAseq data serve as an independent means to verify the accuracy of KozakGAL4 expression patterns for reporting the expression pattern of the targeted gene. Hence, KozakGAL4 alleles serve as an alternative approach to artificial exon-based methods for genes lacking a suitable intron.
We also designed and generated new homology donor vector backbones to facilitate the generation of homology donor constructs. We showed that in vivo linearization of the donor constructs improves the efficiency of incorporation of large constructs (>5kb) using short homology arms (200 bps). Short homology arms make it feasible to commercially synthesize homology donors and minimize the cloning steps in donor construct generation. We have designed new vector backbones that include both gene specific sgRNA(s) and an sgRNA to linearize the homology donor together with the homology arms. This strategy obviates cloning separate sgRNAs and ensures delivery of all the components for targeting a gene in a single plasmid. We show that using this strategy greatly facilitates cloning of the donor constructs and increases the successful transgenesis rate to 80%. These upgrades, together with the KozakGAL4 strategy, will enable efficient targeting of 80% of the conserved fly genes by the Gene Disruption Project.

1021C Nebulous without *white*: annotated long-read genome assembly and CRISPR/Cas9 genome engineering in *D. nebulosa* *Christopher Sottolano*[1], Nicole Revaitis[1], Anthony Geneva[1,2], Nir Yakoby[1,2] 1) Center for Computational and Integrative Biology, Rutgers University, Camden, NJ; 2) Department of Biology, Rutgers University, Camden, NJ

The plethora of morphological diversity among *Drosophila* species presents an opportunity to study the mechanisms underlying the molecular evolution of tissue patterning and morphologies. One of the challenges in investigating these species is that unlike the tremendous number of molecular and genetics tools available for *Drosophila melanogaster* research, many other species do not have sequenced genomes. In addition, the available molecular tools to manipulate genes are very limited. Here, we focus on *D. nebulosa* due to the ease of rearing, its differences in tissue patterning and eggshell morphologies, and outstanding organismal behavior when compared to *D. melanogaster*. As a first step to develop the fly as a new genomic and genetic resource, we generated a high-quality annotated genome assembly of *D. nebulosa*, using PacBio long-read sequencing. We utilized the assembly to successfully disrupt the *white* gene via CRISPR/Cas9, but were unable to integrate the Cas9 gene using homology-directed repair.

Interestingly, unlike many other *Drosophila* species, *D. nebulosa* males null for the *white* gene did not appear to court females. We conclude, gene disruption via CRISPR/Cas9 genome engineering is a useful tool in *D. nebulosa*. However, a simple selectable marker which does not impair vision is needed to replace the commonly used white-eye phenotype.

1022A A nickase Cas9 gene-drive system promotes super-Mendelian inheritance in Drosophila melanogaster. *Sara Sanz Juste*, Victor Lopez del Amo, Valentino Gantz University of California, San Diego

CRISPR gene drive systems can potentially modify an entire population due to their self-propagation capacity, representing a powerful tool to combat public health issues such as mosquito-borne diseases. Gene drive elements bias Mendelian inheritance (50%) towards super-Mendelian rates (~100%), allowing them to spread through populations. Traditional gene drives can replace wildtype alleles by performing DNA double-strand breaks and subsequent homology-directed repair (HDR). When the HDR is not precise, mutations at the target site can generate resistant alleles, preventing a gene drive's efficient spread.

We hypothesize that simultaneous paired-nicks targeting two adjacent DNA regions should generate a staggering double-strand break, and the subsequent DNA repair by HDR will promote the gene-drive super-Mendelian inheritance. Here, we developed a proof-of-concept nickase-based gene drive strategy that enables super-Mendelian inheritance of an engineered allele using Drosophila melanogaster. Additionally, we analyzed the quality of potential resistant alleles formed by our nickase gene-drive system and compared them to the traditional gene drive to explore potential advantages. In fact, we demonstrate for the first time that both nD10A and nH840A promote HDR in the germline using CRISPR gene drives, yielding comparable super-Mendelian inheritance rates to traditional gene drives. Furthermore, the double-nicking nature of this arrangement produced large deletions when using the nickase H840A combined with paired-gRNAs in a PAM-in configuration. This outcome provides potential advantages to target essential genes.

Indeed, our nickase gene-drive design should expand our alternatives for gene drive applications and other germline editing purposes in a broad range of organisms.

1023B High-Resolution Imaging Method with Standardized Conditions Facilitates Reproducible, Spatial, Quantitative Data *Heidi Pipkin*[1], Adam Smiley[2], Andrew Arsham[1] 1) Bemidji State University; 2) University of Minnesota

Collection of reproducible high quality quantitative data on whole flies is challenging, especially in training environments where data collection is distributed among many individuals. Common adjustable light sources for dissecting microscopes create glare, shadow, and irreproducible lighting conditions; microscope-mounted cameras are optimized for low noise and high sensitivity but are often low resolution and can only record 1-2 flies at a time; and only a small portion of the fly is in focus. To address these limitations, we assembled a high-resolution imaging method with fixed lighting, staging, and exposure conditions. Whole flies are frozen and imaged without additional fixation or mounting medium. A 3D printed stage standardizes fly placement and reduces preparation time. Batch collection and computer automation of focus stacking eliminates inter-image and inter-operator variability eliminating the need for complex, irreproducible, and ethically murky image manipulation to adjust color, glare, and similar artifacts. A 50MP full frame digital camera, computer-controlled stepper motor, and focus-stacking software generates a single image of dozens of flies with all ommatidia, bodies, wing veins, and bristles in focus. These standardized composite images facilitate a many-fold reduction in imaging time and precise quantitative comparison of phenotypes involving body and organ color, size, shape, and pattern. Semi-automated data extraction and analysis using ImageJ and R further facilitate reproducibility, code-sharing, and flexibility and reduce the confusion and workflow complexity created by intermediary data products (crops, filters, masks, format conversions, etc.). In addition to being accessible to resource-limited labs, these methods are classroom-friendly and support undergraduate learning outcomes in image analysis, data visualization, computational biology, and statistics.

1024C Developing a High Throughput Drug Induced Phenomics and Transcriptomic Assessment *Robert Courville*, Mahtab Jafari University of California, Irvine, Irvine, CA

Background: *Drosophila melanogaster* may serve as an excellent pre-clinical model system in drug discovery. Developing high throughput platform using *Drosophila* as a model system in drug discovery presents an efficient and cost-effective *in vivo* pre-clinical platform to screen and evaluate therapeutics, at the phenotypic and transcriptomic levels.
Methods: We developed two high throughput screening platforms to evaluate drug induced phenomic changes by two pharmaceuticals with distinct pharmacological properties in an outbred *Drosophila* population: caffeine (a stimulant) and haloperidol (a sedative). The evaluated phenotypes were fecundity and behavioral changes; locomotion, feeding patterns, and sleep. To evaluate fecundity, flies were divided into mating pairs and transferred into vials with removable caps containing the drug/yeast solution. After 24 hours of mating, digital images of vial caps were created using a scanner in groups of 40 by treatment, and the images were uploaded into ImageJ where a custom macro autonomously counted the eggs. To evaluate behavioral changes, we optimized the Activity Recording Cafe (ARC), a machine-vision

system that allows for automated behavioral measurements. Flies were sexed into groups of 30 and habituated in ARC chambers for 24 hours. Machine vision settings were calibrated for optimal frame tracking and data were collected for 24 hours autonomously. Behaviors were measured at days 14 and 20 from eclosion. Transcriptomic analysis will be done at the UC Irvine Genomics High-Throughput facility using heads, ovaries, and testes.
Results: Both drugs impacted fecundity in an age specific manner. Caffeine increased fecundity in days 19-28 ($p<0.05$) but had no impact on fecundity on days 14-18. Haloperidol decreased fecundity in days 14-18 ($p<0.05$) and had no impact on fecundity in other ages. Caffeine increased fly activity and feeding relative to controls ($p<0.05$), and decreased sleep ($p<0.05$). Haloperidol showed decreased activity at both ages ($p<0.05$), but no significant differences in feeding or sleep. All phenomic assays are currently being replicated. Transcriptomic analysis is in progress. The efficiency (time and cost) of these high-throughput assays will be compared with conventional assays and the data will be presented on the poster.
Conclusion: The phenomic data show that the high throughput screening platforms were sensitive enough to detect drug induced phenomic changes.

1025A A novel transposable element based authentication protocol for *Drosophila* cell lines *Daniel Mariyappa*[1], Douglas Rusch[1], Shunhua Han[2], Arthur Luhur[1], Danielle Overton[1], David Miller[1], Casey Bergman[2], Andrew Zelhof[1] 1) Indiana University; 2) University of Georgia

Drosophila cell lines are used by researchers to investigate various cell biological phenomena. It is crucial to exercise good cell culture practice. Poor handling can lead to both inter- and intraspecies cross-contamination. Prolonged culturing can lead to introduction of large- and small-scale genomic changes. These factors, therefore, make it imperative that methods to authenticate *Drosophila* cell lines are developed to ensure reproducibility. Mammalian cell line authentication is reliant on short tandem repeat (STR) profiling, however the relatively low STR mutation rate in *D. melanogaster* at the individual level is likely to preclude the value of this technique. In contrast, transposable elements (TE) are highly polymorphic among individual flies and abundant in *Drosophila* cell lines. Therefore, we investigated the utility of TE insertions as markers to discriminate *Drosophila* cell lines derived from the same or different donor genotypes, divergent sub-lines of the same cell line, and from other insect cell lines. We developed a PCR-based next-generation sequencing protocol to cluster cell lines based on the genome-wide distribution of a limited number of diagnostic TE families. We determined the distribution of five TE families in S2R+, S2-DRSC, S2-DGRC, Kc167, ML-DmBG3-c2, mbn2, CME W1 Cl.8+, and OSS *Drosophila* cell lines. Two independent downstream analyses of the NGS data yielded similar clustering of these cell lines. Double-blind testing of the protocol reliably identified various *Drosophila* cell lines. In addition, our data indicate minimal changes with respect to the genome-wide distribution of these five TE families when cells are passaged for at least 50 times. The protocol developed can accurately identify and distinguish the numerous *Drosophila* cell lines available to the research community, thereby aiding reproducible *Drosophila* cell culture research.

1026B Genetic barcoding for single cell transcriptomics and population behavioral assays Jorge Blanco Mendana, Margaret Donovan, Benjamin Auch, *Daryl Gohl* University of Minnesota, Minneapolis, MN

Advances in single cell sequencing technologies have provided novel insights into the dynamics of gene expression throughout development, been used to characterize somatic variation and heterogeneity within tissues and are currently enabling the construction of transcriptomic cell atlases. However, despite these remarkable advances, linking anatomical information to transcriptomic data and positively identifying the cell types that correspond to gene expression clusters in single cell sequencing data sets remains a challenge. We are developing a straightforward genetic barcoding approach that takes advantage of the powerful genetic tools available in Drosophila to allow *in vivo* tagging of defined cell populations. This method, called Targeted Genetically-Encoded Multiplexing (TaG-EM), involves inserting a DNA barcode just upstream of the poly-adenylation site in a Gal4-inducible UAS-GFP construct, so that the barcode sequence can be read out during single cell sequencing, labeling a cell population of interest. By creating many such independently barcoded fly strains, TaG-EM will enable a number of potential applications that will improve the quality and information content of single cell transcriptomic data. Potential applications of this method include positive identification of cell types in cell atlas projects, barcoding of experimental timepoints, conditions, or replicates, and detection and elimination of multiplets through detection of multiple barcodes. Furthermore, we demonstrate that the barcodes from TaG-EM fly lines can be read out using next-generation sequencing to facilitate population-scale behavioral measurements. Thus, TaG-EM will enable many types of large-scale behavioral screens in addition to improving the ability to reliably annotate cell atlas data, expanding the scope of single cell transcriptomic experiments, and improving the robustness of such data by facilitating inclusion of replicates.

1027C RNA viral metagenomics of 100-year-old *Drosophila melanogaster* museum specimens *Alexandra Keene*, Mark Stenglein Colorado State University, Fort Collins, CO

Paleogenomic sequencing has enabled the reconstruction of extinct genomes and shed light on ancient host-pathogen interactions. But sequencing has focused for the most part on ancient DNA rather than ancient RNA, in part because

of a belief that RNA is fragile and short-lived. In this study, I experimentally assessed the stability of single and double-stranded RNA over months in dried insects. I also optimized methods for non-destructive extraction of RNA from pinned *D. melanogaster* museum specimens that had been collected over the past 120 years. I was particularly interested in reconstructing the historical dynamics of *D. melanogaster* and galbut virus, which is an exceptionally successful persistent virus in current fly populations. Galbut virus is a three-segmented partitivirus that is ubiquitous in wild *D. melanogaster* populations: its average global prevalence is higher even than that of *Wolbachia*. This high degree of success is attributable to galbut virus being biparentally vertically transmitted with near 100% efficiency, which should drive the virus to 100% prevalence in wild populations. But multiple lines of evidence indicate that some flies are resistant to infection and this virus and host may be co-existing in an uneasy equilibrium. To understand how long galbut virus has been infecting *D. melanogaster* and how its sequence might have changed over the past century, I obtained specimens ranging from 15-120 years from entomological collections across the United States. I obtained fragmented but detectable RNA from these old specimens and was able to amplify galbut virus sequences using short-range RT-qPCR from century-old flies. I am extending and confirming this finding using shotgun RNA sequencing to fully reconstruct the virome of these historic specimens. This indicates that this currently successful virus has infected *D. melanogaster* since the dawn of *Drosophila* research, and supports the conclusion that ancient dried insect specimens are a source of usable RNA for paleogenomic studies.

1028A PECAn, a pipeline for image processing and statistical analysis of complex mosaic 3D tissues Michael Baumgartner, *Remi Logeay*, Paul Langton, Alex Mastrogiannopoulos, Eugenia Piddini University of Bristol

Investigating organ biology requires sophisticated methodologies to induce genetically distinct clones within a tissue. Microscopic analysis of such samples produces information-rich 3D images. However, the 3D nature and spatial anisotropy of patches of cells makes sample analysis challenging and slow and limits the amount of information that can be extracted manually. Here we have developed a pipeline for image processing and statistical data analysis which automatically extracts sophisticated parameters from complex multi-genotype 3D images. The pipeline includes data handling, machine-learning-enabled segmentation, multivariant statistical analysis, and graph generation. This enables researchers to run rigorous analyses on images and videos at scale and in a fraction of the time, without requiring programming skills. We demonstrate the power of this pipeline by applying it to the study of Minute cell competition. We find an unappreciated sexual dimorphism in Minute competition and identify, by statistical regression analysis, tissue parameters that model and predict competitive death.

1029B MARRVEL and ModelMatcher: Online resources to facilitate cross-disciplinary collaborations between scientists, clinicians and beyond *Shinya Yamamoto*[1,2], J Michael Harnish[1,2], Lucian Li[2,3], Seon-Young Kim[2,3], Sanja Rogic[4,5], Guillaume Poirier-Morency[4,5], Kym M Boycott[6], Philip Hieter[4], Paul Pavlidis[4,5], Michael F Wangler[1,2], Hugo J Bellen[1,2], Zhandong Liu[2,3], Undiagnosed Diseases Network 1) Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX, USA; 2) Jan and Dan Duncan Neurological Research Institute, Texas Children's Hospital, Houston, TX, USA; 3) Department of Pediatrics, Baylor College of Medicine, Houston, TX, USA; 4) Michael Smith Laboratories, University of British Columbia, Vancouver, BC, Canada; 5) Department of Psychiatry, University of British Columbia, Vancouver, BC, Canada; 6) Children's Hospital of Eastern Ontario Research Institute, University of Ottawa, Ottawa, ON, Canada

Progress in 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 important 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 have been lagging. To fill this gap, we have been developing MARRVEL (https://marrvel.org) and ModelMatcher (https://www.modelmatcher.net), two informatic tools that allow scientists to effectively gather information about a gene of interest in diverse species including human and to identify clinical collaborators on a global scale, respectively. Using these tools, *Drosophila* researchers can quicky gather information about the human and other model organism orthologs of their genes of interest and identify potential clinical and scientific collaborators around the world. While there is a lot of value in pursuing fundamental scientific questions that do not have obvious or immediate clinical value, an active involvement in a clinical project may help increase the significance of a specific research project, and increase the impact of the basic scientific work.

1030C A toolkit to generate interconvertible overexpression *Drosophila* transgenes *Luis Alberto Baena Lopez*[1], Franz Wendler[1], Sangbin Park[2], Claire Hill[1], Alessia Galasso[1], Kathleen Chang[2], Iman Awan[1], Yulia Sudarikova[1], Mar Bustamante-Sequeiros[1], Sichen Liu[1], Ethan Seung[1], Gabrielle Aisabonoko[1], Seung Kim[2] 1) University of Oxford; 2) Department of Developmental Biology, Stanford University School of Medicine

Three independent binary systems for conditional gene expression (Gal4/UAS; LexA/LexAop; QF/QUAST) have greatly

aided the genetic investigations using *Drosophila* as a model organism. However, each of these inducible gene expression systems requires the independent generation of non-interchangeable fly repositories. To alleviate this important logistic disadvantage and the need of triplicating transgene repositories for combinatorial experiments, we developed a modular vector that contains the regulatory elements for all three binary systems. This enables the separate or simultaneous overexpression of new transgenes through any of the available transcriptional factors (Gal4-, LexA- or QF), thus offering new experimental possibilities for *in vivo* cDNA overexpression (robust RNAi downregulation needs to be individually tested). Additionally, each transcriptional regulatory element (UAS, LexAop, QUAS) was flanked by two different but compatible recombination sites that facilitate their elimination from the genome with spatial and temporal control upon exposure to adequate recombinases. This allows the efficient generation of genetic mosaics within somatic cells as well as the elimination in the germline of specific gene-activating sequences. These unique features of our genetic toolkit provide unprecedented experimental possibilities and logistical advantages such as the generation of new fly lines with a subset of gene regulatory elements of interest upon excising the unwanted in a common precursor strain containing all of them.

1031A Comprehensive Resource for the Drosophila 4th Chromosome *Michael Stinchfield*[1], Mary Jane O'Connor[2], Brandon Weasner[3], Bonnie Weasner[3], Justin Kumar[3], Kevin Cook[3], Michael O'Connor[2], Stuart Newfeld[1] 1) School of Life Sciences, Arizona State University, Tempe, AZ ; 2) Dept. Genetics, Cell Biology and Development, Univ. Minnesota, Minneapolis, MN; 3) Dept. Biology, Indiana University, Bloomington, IN

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 105 genes (79 protein coding and 26 noncoding RNA). 74% of the protein coding genes have human homologs and 68% of these have a disease association. For example Eyeless belongs to the PAX/RAX family where inherited loss of PAX6 leads to Aniridia and somatic loss of RAX2 leads to age-related macular degeneration. A complete understanding of metazoan biology and thus human health requires the examination of these genes. To advance this effort ASU, IU and UMN will collaborate to create a resource facilitating the analyses of genes on the 4th. The resource will contain stocks divided into several collections. 1. FRT101F with a CRISPR mutation for loss of function and MARCM studies. 2. Conversion of protein coding genes with an existing MIMIC or CRIMIC to T2A.GAL4 and eGFP for protein traps and gain of function studies. 3. Gain of function stocks with a UAS.fly cDNA for each protein coding gene. 4. Gain of function stocks with a UAS.human cDNA for the two closest human homologs for each conserved protein coding gene. 5. Auxiliary chromosomes and balancers. Progress on all collections will be described. This resource will be made available through BDSC to all qualified investigators.

1032B Multiscale, multi-perspective imaging assisted robotic microinjection of *Drosophila melanogaster* embryos *Andrew D. Alegria*[1], Amey S. Joshi[1], Kunpeng Liu[1], Jorge Blanco Mendana[2], Benjamin Auch[2], Daryl M. Gohl[2,3], Suhasa B. Kodandaramaiah[1,4,5] 1) University of Minnesota Twin-Cities, Department of Mechanical Engineering, Minneapolis, MN; 2) University of Minnesota Twin-Cities, University of Minnesota Genomics Center, Minneapolis, MN; 3) University of Minnesota Twin-Cities, Department of Genetics, Cell Biology, and Development, Minneapolis, MN; 4) University of Minnesota Twin-Cities, Department of Biomedical Engineering, Minneapolis, MN; 5) University of Minnesota Twin-Cities, Department of Neuroscience, Minneapolis, MN

Microinjection is an important technique that has enabled transgenesis and targeted mutagenesis in *Drosophila*, as well as the wide array of methods and genetic toolkits which depend on transgenesis. However, microinjection remains a labor-intensive and highly specialized manual procedure, which makes it a critical bottleneck in the field and thus ripe for automation. Here, we present a computer-guided robot that automates the targeted microinjection of *Drosophila melanogaster* embryos. The robot uses a series of cameras and microscopes to image a petri-dish containing embryos at multiple magnifications and perspectives. This imaging is combined with a machine learning algorithm and computer vision algorithms to pinpoint a location on the embryo for targeted microinjection with microscale precision. We demonstrate the ability of the robot to successfully microinject *Drosophila melanogaster* embryos. Results obtained indicate that the robot can significantly increase throughput as compared to manual microinjection since approximately in 1 hour the robot can inject upwards of 200 embryos. Since microinjections are performed in embryos on the petri-dish, the robot further eliminates the preparatory steps such as the dechorionation and placing of the embryos on a glass slide prior to microinjection. Further, the robot is able to maintain survivability rates comparable to that of manual microinjection with survival rates upwards of 50%. We have demonstrated robust and reproducible transposon and PhiC31-mediated transgenesis with automated robotic microinjection of embryos directly on petri-dishes. This microinjection robot provides a means to achieve scalability as additional systems can be built at relatively low cost ($13,000) allowing parallelization of the injection process. In the future, we anticipate the robot can be used to perform high throughput microinjections and potentially enable new types of injection-intensive experiments.

1033C Making Hox Gene-specific Drivers Using a Modified Trojan-exon Strategy *Fengqiu Diao*, Benjamin White National Institute of Mental Health, NIH, Bethesda, MD

Hox family transcription factors are selectively expressed in distinct segments along the neuraxis of the *Drosophila* central nervous system. This regional specificity of expression, together with the functional importance of Hox gene-expressing neurons in motor behaviors, makes Hox genes attractive candidates for gene- and region-specific targeting methods. The "Trojan-exon" strategy has been broadly successful in producing gene-specific drivers, but producing such drivers for developmentally important transcription factors, such as Hox genes, has been problematic (Diao F et al, 2015). This may be because these transcription factors are particularly sensitive to truncation of the protein translated from the allele containing the Trojan exon. Truncation necessarily lowers the level of transcription factor expression, but may also result in dominant-negative translation products that suppress transcriptional activity. To address these problems, we have developed a novel type of Trojan exon that incorporates split inteins. The protein fragments derived from a gene whose translation is normally interrupted by the Trojan exon will thus be re-ligated into a full-length active gene product, while at the same time allowing for expression of Gal4 or Split Gal4 components. Using this method, we have successfully made Gal4 drivers for the *labial* (*lab*), *Proboscipedia* (*Pb*), *deformed* (*dfd*), *Antenapedia* (*Antp*), *Ultrabithorax* (*Ubx*) and *Abdominal-A* (*Abd-A*) genes. In addition, we have made Split Gal4 hemidrivers for the *lab*, *dfd*, *Abd-A* and *Sex Combs-reduced* (*Scr*) genes. Gal4-driven UAS-reporter expression in the CNS is consistent with the known expression patterns of these genes. In addition, functional manipulation of previously characterized cell types yields the anticipated results. *Dfd* and other Hox genes play well-established roles in motor function, and we find that our *Dfd*- and *Scr*-specific hemidrivers express in glutamatergic neurons, including putative motor neurons. Consistent with this, suppression of neurons targeted by Dfd-Gal4 at the larval stage impairs feeding by preventing the mouth-hooks from being elevated. Conversely, stimulation of these neurons leaves the mouth-hooks constitutively elevated. In adults, stimulation of neurons in the Dfd-Gal4 pattern also alters feeding behavior by eliciting vigorous proboscis extension. Our results suggest that the new Hox-gene specific drivers express faithfully in their respective cell types and will be useful in developmental, physiological, and neural circuit-mapping studies.

1034V Towards a novel method for cryopreservation via embryonic nuclear transplantation in Drosophila *Troy Louwagie*[1], Grace Kringle[2], Jorge Blanco Mendana[3], Lindsey Gengelbach[3], Benjamin Auch[3], Daryl Gohl[3,4], Allison Hubel[1,2,5] 1) University of Minnesota- Twin Cities, Department of Mechanical Engineering, Minneapolis, MN; 2) University of Minnesota-Twin Cities, ATP-Bio, Minneapolis, MN; 3) University of Minnesota-Twin Cities, University of Minnesota Genomics Center, Minneapolis, MN; 4) University of Minnesota-Twin Cities, Department of Genetics, Cell Biology, and Development, Minneapolis, MN; 5) University of Minnesota-Twin Cities, Department of Biomedical Engineering, Minneapolis, MN

Cryopreservation of *Drosophila melanogaster* has been an area of interest since the 1990s when methods of embryo vitrification protocols were introduced by Stepnokus et al. (1990) and Mazur et al. (1992). However, there has still not been widespread adoption of these methods due to the difficulty of the protocols. Recent publications from Zhan et al. (2021) and Asaoka et al. (2021) have shown successful development of an alternative vitrification methods for cryopreservation of embryos and a novel cryopreservation method based on transplantation of cryopreserved primordial germ cells, respectively. We are testing an alternative method that involves the slow cooling of embryonic nuclei and regeneration of stocks by embryonic nuclear transplantation (ENT). Using confocal Raman spectroscopy is beneficial for label-free imaging of nuclei and the distribution of water and CPA. Osmolyte solutions consisting of sucrose, glycerol, and other buffering agents that aid in the isolation of Drosophila nuclei act as quality CPAs in the slow-cooling cryopreservation process. Studies at 4°C show that certain CPAs, such as glycerol, can permeate the nuclear membrane while other CPAs, like sucrose, cannot penetrate the nuclear membrane. Using a controlled rate temperature-cooling stage, we can image the nucleus at -50°C with varying cooling rates and nucleation temperatures. We are also using an RT-qPCR assay for *hsp70* induction to monitor nuclei health and viability throughout the isolation, cryopreservation, and recovery process. This functional assay will aid in determining the optimal parameters of Drosophila cryopreservation with the use of a differential evolution (DE) algorithm. The DE algorithm uses experimental observations (CPA composition, cooling rates, nucleation temperatures, and post thaw viability) to modify existing population vectors and predicts solutions that may be more favorable. This DE algorithm could also be applied to other Drosophila cryopreservation methods to optimize CPA and freezing parameters. Finally, we have been attempting to create clones or chimeric embryos derived from isolated nuclei using ENT. ENT has previously been demonstrated in *Drosophila* and avoids issues associated with the impermeability of Drosophila embryos to cryoprotectants. We report progress in developing this novel method which has the potential to provide additional options for the practical cryopreservation of Drosophila stocks.

1035V Development of a fly model to probe the functions of inorganic polyphosphates *Sunayana Sarkar*[1], Harsha Sharma[1], Jayashree Suresh Ladke[2], Rashna Bhandari[2], Manish Jaiswal[1] 1) TATA Institute of Fundamental Research, Hyderabad, India; 2) Centre for DNA Fingerprinting and Diagnostics, Hyderabad, India

Inorganic polyphosphate (polyP), which consists of chains of orthophosphate residues of varying chain lengths, is found in all living organisms alongside other phosphate-rich biomolecules such as ATP and inositol phosphates. The biological functions of polyP have been extensively studied in prokaryotes and unicellular eukaryotes, however, their functions in

metazoans are largely underexplored. In fact, polyP has been referred to as a molecular fossil. In recent years polyP has been shown to be implicated largely in blood clotting and bone mineralisation in mammals. Moreover, several other functions of polyP are predicted due to its presence in various cell types and its specific binding with numerous 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 embryogenesis. Further, through phylogenetic analysis, we characterised Prune as a putative exopolyphosphatase. Indeed, we observed significantly higher levels of polyP in prune mutants as compared to wildtype flies. Prune is a mitochondrial protein known to hydrolyze cAMP. Mutations in prune are linked to a variety of processes such as mitochondrial biogenesis, eye pigmentation and neurodegeneration. Based on our data, we surmise that prune mediated regulation of polyP may attribute to some of the prune phenotypes and will help understand polyP biology in metazoans.

1036V A split-Gal4 system that is repressible by Gal80 *Ben Ewen-Campen*[1], Haojiang Luan[3], Jun Xu[1], Tanuj Thakkar[1], Benjamin White[3], Norbert Perrimon [1,2] 1) Harvard Medical School, Boston, MA; 2) HHMI; 3) National Institute of Mental Health, NIH, Bethesda, MD

Most of the Gal4 drivers that are commonly used in *Drosophila* research are not solely expressed in the cells- or tissues-of-interest, but also in additional, often unwanted cells or tissues. To overcome this relative lack of specificity, the split-Gal4 intersectional technique was developed, which has successfully been used to create extraordinarily specific driver lines, including many expressed in only a single cell-type. However, unlike native Gal4, existing split-Gal4 tools cannot be repressed by the Gal80 repressor or any other commonly-used repressor, and therefore cannot be temporally controlled. Here, we present a split-Gal4 system which is fully repressible by Gal80 *in vivo*. Based on a split-intein, this system drives transgene expression at levels indistinguishable from the original split-Gal4 system, and combines the cell-type specificity of split-Gal4 with the temporal control of native Gal4/Gal80. In addition to creating highly specific genetic driver lines, we show that this system can also be used to systematically map single cell RNAseq clusters to anatomy.

1037V Efficient allelic conversion by homologous chromosome-templated repair in *Drosophila* somatic tissues *Annabel Guichard*, Sitara Roy, Sara Juste, Marketta Sneider, Ankush Araudkar, Zhiqian Li, Ehan Bier Univ California, San Diego, CA

Recent advances in Cas9-based genome editing offer broad applications in research, agriculture and medicine. These techniques typically entail insertion of exogenous gene sequences provided by a donor template into specific genomic sites following Cas9-mediated DNA cleavage. Here, we demonstrate efficient somatic allelic conversion wherein cleavage-resistant sequences present on one chromosome correct a targeted cleavage-sensitive allele at the corresponding site on the homologous chromosome. Using a set of allelic combinations of the *white* locus of *Drosophila,* we show successful allelic conversion as revealed by quantifiable red eye clones produced by Cas9-dependent cleavage, or by its nickase variants Cas9D10A and Cas9H840A. Repair phenotypes elicited by Cas9 versus nickases differ substantially in several respects, including efficiency, production of cleavage-resistant alleles through the NHEJ mutagenic pathway and developmental timing. We show that allelic correction does not strictly require long-range chromosomal pairing, and that sequence homology alone can promote such repair from a non-homologous location in the genome. If generalized to human cells, this simple approach could be adapted to correct a wide array of dominant or trans-heterozygous genetic conditions offering novel gene therapy strategies.

1038V A method to estimate the frequency of chromosomal rearrangements induced by CRISPR/Cas9 multiplexing in Drosophila *Bruce Reed*, William Ng, Richard Do, Brittney Lato, Emily Baker, Mackenzie Vallee University of Waterloo, Waterloo ON, CANADA

Using CRISPR/Cas9 to simultaneously induce mutations in two or more target genes, commonly referred to as multiplexing, may result in chromosomal rearrangements such as inversions or translocations. While this may be undesirable in some contexts, the ability to recover chromosomal rearrangements targeted to specific sites in the genome is potentially a powerful tool. The frequency of chromosome rearrangements induced by CRISPR/Cas9 multiplexing, however, remains unknown in Drosophila. To estimate the frequency of chromosome rearrangements induced by multiplexing, we developed a self-selecting screening system using Drosophila stocks that carry an autosomal pericentric inversion in what is known as the autosynaptic form. All progeny of normal females crossed to males of these autosynaptic stocks are lethal due to excessive aneuploidy. If an inversion is induced within the female germline, however, and if this inversion is analogous to the inversion in the male autosynaptic line, then it is possible to recover progeny in which aneuploidy is reduced and viability is restored. Using this self-selection screening method, we were able to estimate the frequency of viable progeny from females having germline expression of active Cas9 together with ubiquitously expressed sgRNAs targeted to desired inversion breakpoints. Salivary gland polytene chromosome analysis, PCR, and sequencing confirmed the recovery of chromosome breakpoints induced between the two sgRNA target sites. Overall, we demonstrate that CRISPR/Cas9 multiplexing can induce chromosomal rearrangements in Drosophila.

In general, the generation of a pericentric inversion within a female germline using CRISPR/Cas9 multiplexing varied depending on the choice of sgRNAs and occurred less than once for every 100 germlines tested. This frequency was also found to be comparable to using FLP/FRT site specific recombination. In using this particular system, we conclude that the induction of chromosomal rearrangements associated with CRISPR/Cas9 multiplexing is not a high frequency event.

1039V Identification of *Drosophila* new genes using machine learning *Gabriel Goldstein*[1,2], Maria Vibranovski[1,3], Yong Zhang[2] 1) Universidade de São Paulo, USP, São Paulo, Brazil; 2) Chinese Academy of Sciences, CAS, Beijing, China; 3) Arizona State University, ASU, Tempe, USA

New genes are defined by their presence in a taxon and absence in sibling taxa. These genes have great biological importance and are involved in processes of high selective pressure, being expressed in tissues such as brain and testis. There are a variety of genetic mechanisms that can lead to the generation of new genes, such as duplications and retrotranspositions for example, but most new genes are derived from duplications. The exact functions of these genes in organisms are still being studied, but some work has already shown a relationship with the resolution of sexual conflicts, for example. Despite this, there are a number of biological characteristics that are known to be different between new and old genes. An example of this is the expression profile of these groups, since new genes are mostly expressed in male gametogenesis and old genes are broadly expressed. The main gene dating method for identifying new genes uses synteny, which is the phenomenon of conservation of the order and gene content of a region in the genome that occurs in related species, and parsimony when comparing genomes of related species to date all genes of a focal species. Despite the accuracy of the method, it is extremely dependent on the assembly and annotation of the genome of interest, which limits its application to model species that have a manual and curated annotation. With these facts in mind, we propose in this work a method of identifying new genes that uses biological information to separate new and old genes through the use of machine learning. Machine learning algorithms are those able to change with experience and are able to identify complex patterns and identify classes from a variety of information. With this, we trained a model with the random forest algorithm in the model species *Drosophila melanogaste*r and obtained 0.508 precision and 0.718 recall with generated data. In addition, we identified the 1523 new genes of *D. pseudoobscura* using the existing method so that we can use this species as a second control point for our method.

1040V REDfly: The Regulatory Element Database for *Drosophila* and other insects *Soile V. E. Keränen*[1], Andrew E. Bruno[2], Marc S. Halfon[3,4,5] 1) None; 2) Center for Computational Research, University at Buffalo-State University of New York, Buffalo, NY; 3) Departments of Biochemistry, Biomedical Informatics, and Biological Sciences, University at Buffalo-State University of New York, Buffalo, NY; 4) NY State Center of Excellence in Bioinformatics & Life Sciences, Buffalo, NY; 5) Department of Molecular and Cellular Biology and Program in Cancer Genetics, Roswell Park Comprehensive Cancer Center, Buffalo, NY

REDfly is a comprehensive knowledgebase of experimentally-validated *cis*-regulatory modules (CRMs) and transcription factor binding sites (TFBSs) for *Drosophila* and other insects, including the malaria mosquitoes *Anopheles gambiae* and *Aedes aegypti* and the beetle *Tribolium castaneum*. The database contains data on more than 53,500 regulatory sequences and almost 2700 TFBSs or their variants. Our goal is to include all functionally tested or predicted sequences, both with and without observable regulatory activity, 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 depending on the experimental evidence underlying their identification: reporter gene analysis, deletion of sequences from the genome, or predictions based on genomic assays such as ChIP-seq, ATAC-seq, and computational modeling. Finer-tuned filtering based on evidence type is possible through the ”advanced search” interface. An additional key REDfly feature is its extensive and searchable expression pattern annotation for each CRM’s activity using structured anatomy, staging, and biological process ontologies. Recently, REDfly has started to curate silencers, which inhibit gene transcription, in addition to the existing curation of both tested and predicted enhancers. Also new is a ”species” page that provides summaries of the data included for each species curated by REDfly, with BLAT and in silico PCR interfaces for each annotated genome. All REDfly code has been made open source. Future plans include updating REDfly’s search and download features to better serve our users, and providing a customized JBrowse interface for improved visualization and integration of REDfly data. REDfly is freely accessible at http://redfly.ccr.buffalo.edu and can be followed on Twitter at @REDfly_database.

1041V Proteomic mapping of organ secretomes using in vivo proximity labeling *Justin A. Bosch*[1], Pierre M. J. Beltran[2], Cooper Cavers[1], Thai LaGraff[1], Randy Melanson[2], Steven Carr[2], Norbert Perrimon[1,3] 1) Department of Genetics, Blavatnik Institute, Harvard Medical School, Boston, MA; 2) Broad Institute, Cambridge, MA; 3) Howard Hughes Medical Institute

Secreted proteins regulate essential biological processes and are attractive for developing therapeutics. Due to their low abundance in extracellular fluids, discovering the complete set of secreted proteins (the “secretome”) - as well as their tissue of origin – can be extremely challenging. To address this, we developed a proteomic method to label proteins in specific organs in *Drosophila* larvae using the biotin ligase TurboID, and subsequently enrich and identify biotinylated

proteins in the hemolymph. Using this tool, we constructed a secretome map of 540 proteins from 10 major cell types (e.g. fat body, muscle). In addition to identifying most known blood proteins (e.g. Lsp2, apolpp), we discovered hundreds of uncharacterized proteins, in many cases derived from a single cell type. Furthermore, while most blood proteins originate from the fat body, we identify secreted proteins from less appreciated cell-types, such as oenocytes and glia. Using CRISPR/Cas9 knock-in flies to validate selected tissue-specific uncharacterized proteins, we found that most were expressed in the presumed tissue and some were detected in circulation by western blot. One protein, CG6867, the single fly homologue of human Olfactomedin proteins, is specifically expressed in somatic muscle and secreted into larval hemolymph. Interestingly, CG6867 accumulates on the basal surface of most tissues (e.g. imaginal discs), suggesting CG6867 may be a novel circulating basement membrane protein. Our secretome map will serve as a resource to investigate blood protein function, discover organ communication factors, and compare with homologues of human biomarkers.

1042V Importance of cell-cycle and cell-sex correction in single-cell analysis: unmasking novel target genes of the Hedgehog pathway *Nicholas Everetts*, Melanie Worley, Riku Yasutomi, Nir Yosef, Iswar Hariharan University of California, Berkeley

Single-cell transcriptomics is an unparalleled technique for obtaining a holistic understanding of biological systems, including tissue composition, organ development, and cellular differentiation. Many computational packages offer default end-to-end analysis for single-cell RNA sequencing (scRNAseq), performing common tasks such as dimensionality reduction, clustering, and differential expression testing. Furthermore, when processing multiple samples, there are a plethora of tools available for integrating datasets. While much attention has been given to the importance of batch correction, fewer studies examine biological covariates within datasets that can mask important signals and decrease statistical power. We examined the how strong biological signals can obscure interesting biology by examining the adult muscle precursor cells (AMPs) of the *Drosophila* wing-imaginal disc, a population of cells that develop into all adult flight muscles but exhibit high transcriptional homogeneity during larval development. We show that by using scRNAseq analysis tools with default settings, cell clusters are primarily split by two biological features: cell-cycle status and the biological sex of the donor fly. This stratification not only hinders the classification of AMPs into their canonical cell types, the “direct” and “indirect” AMP subpopulations, but also obscures the discovery of new biological signals and interactions by separating otherwise transcriptionally-similar cells. Cell-cycle and cell-sex signatures are observable within the latent space generated by dimensionality reduction, enabling *post hoc* correction by identifying the latent dimensions that correlate with markers of proliferation and biological sex. By removing these confounded latent dimensions from downstream processing, cell-type classification of AMPs is improved and the Hedgehog pathway is unmasked as an important signaling pathway for the proper development of adult flight muscles. With our analysis, we identified and validated two novel Hedgehog-signaling targets, *Neurotactin* and *midline*, within the AMPs. Additionally, confounding covariates can be supplied to the deep-learning single-cell software *scvi-tools*, taking advantage of its powerful integration models while suppressing unwanted biological signals. Our research highlights the importance of identifying and correcting for unnecessary biological signals in addition to technical effects, and provides easily-implemented solutions for removing signals such as cell cycle and cell sex.

1043V Characterization of shock wave effects in syncytial embryos of *Drosophila melanogaster* using fluorescent nanoparticles *Daniel Tapia Merino*[1,2,5], Achim Max Loske Mehling[3,5], Gabriel Ramos Ortiz[4], Juan Rafael Riesgo Escovar[2,5] 1) Maestria en Ciencias (Neurobiologia), INB, Queretaro, Mexico; 2) Instituto de Neurobiologia (INB), Queretaro, Mexico; 3) Centro de Fisica Aplicada y Tecnologia Avanzada (CFATA), Queretaro, Mexico; 4) Centro de Investigaciones en Optica, A.C. , Guanajuato, Mexico; 5) Universidad Nacional Autonoma de Mexico Campus Juriquilla, Queretaro, Mexico

We employed fluorescent nanoparticles as fiducial markers to evidence the insertion of material into the early syncytial embryo of *Drosophila melanogaster*, using underwater shock waves. These mechanical waves generate microjets that, in principle, perforate the outer layers of the embryos (chorion and vitelline membranes) as well as the embryonic plasma membrane. A convenient shock wave pressure profile was found, to guarantee the survival and growth of a reasonable percentage of the treated embryos to become viable, fertile adults. Our results reveal that fluorescent nanoparticles of different sizes and composition, added to the incubation medium, upon shock wave exposure, are able to penetrate the embryonic layers, and stay inside the fly tissues. These results could lead to diverse biological applications in a few simple steps.

1044V OligoY: a pipeline for the design of repetitive oligopaint probes for the Y Chromosome *Isabela Almeida*[1], Henry Bonilla Bruno[1], Mara Pinheiro[1], Amanda Luvisotto[1], Antonio Carvalho[2], Maria Vibranovski[1] 1) Universidade de São Paulo, USP, São Paulo/SP, Brazil; 2) Universidade Federal do Rio de Janeiro, UFRJ, Rio de Janeiro/RJ, Brazil

Studies with the Y chromosome offer a series of obstacles, one of them being the heterochromatic and highly repetitive state of this structure, leading to difficulties in assembling scaffolds and contigs and, thus, in a lack of final assembled sequences for it. The Y chromosome of *Drosophila melanogaster*, the model organism used in this research, has an estimated size of 41Mb of repeat-rich sequences, but only 10% of them are assembled in the most recent genome

release. In contrast, the protocol for designing probes used in full chromosome fluorescent labeling experiments (FISH Oligopaint) does not include repetitive sequences to avoid off-target hybridization. For this reason, there are currently less than 1500 oligopaint probes for this Y chromosome model, which is a value at least ten times smaller when compared to the one observed for other chromosomes of the same species. Furthermore, this amount is insufficient to carry out FISH Oligopaint assays efficiently. The main objective of this research is to develop a pipeline that allows the design of oligopaint probes for the Y chromosome off any species of interest. The final pipeline includes the use of open-source and existing tools in Bioinformatics, identification of sequences unique to the chromosome of interest, guarantees the user the autonomy to choose parameters and effectively uses repetitive sequences unique to the target chromosome to design probes, therefore maximizing overall efficiency of cytogenetic experiments. After extensive tests and validations *in silico* and *in situ*, it was verified that the application of the developed pipeline, OligoY, allows staining the Y chromosome without generating off-target signal, despite the use of repetitive sequences for oligopaint probe design. In addition, some of the OligoY pipeline strategies' can be used in other chromosomes to address gaps in repeat-rich regions that otherwise would not be stained.

1045V A Bibliometric Analysis of Somatic Mutation and Recombination Tests of *Drosophila melanogaster* *Ghada Tagorti*, Bülent Kaya Akdeniz University, Antalya, Turkey.

Somatic mutation and recombination tests (SMARTs) are accurate and low-cost assays performed to assess the genotoxic potential of multiple pollutants by using *Drosophila melanogaster* as a model organism. The present study attempts to understand the current research situation and knowledge base in SMART assays research. To accomplish this, data were extracted from the Web of Science Core Collection from 1984 to 2020, to be analyzed by HistCite, Biblioshiny (RStudio), VOSViewer, and CiteSpace. Results have shown an increase in publications within the last ten years, with an annual rate of 14.4 articles. A total of 392 records involving 35 countries were published in 96 sources, mainly from Brazil (*n* = 106; 27.04%), Spain (*n* = 69; 17.60%), and Turkey (*n* = 66; 16.84%). The *Federal University of Uberlândia* ranked first in terms of publications (*n* = 39; 9.95%). The *Mutation Research-Genetic Toxicology and Environmental Mutagenesis* journal had the highest total global citation score per year. Based on document co-citation, five large research clusters were identified. The 'nanoparticles' cluster was the youngest research frontier. The top-cited documents focused on SMARTs protocols, statistical tests, and proposed strains to improve the scoring. With this study, the evolution of SMARTs research trends over years was demonstrated. However, international collaboration should be more encouraged and supported.

1046A The Genomics Education Partnership: Teaching and Research Opportunities *Raffaella Diotti*[1], Cindy Arrigo[2], Martin G. Burg[3], Justin R. DiAngelo[4], Sarah C. R. Elgin[5], Christopher E. Ellison[6], Christopher J. Jones[7], Jennifer Kennell[8], Judith Leatherman[9], Wilson Leung[5], David Lopatto[10], Vida Mingo[11], Laura K. Reed[12], Chinmay P. Rele[12], Katie M. Sandlin[12], Jamie Siders[13], Norma Velazquez-Ulloa Velazquez-Ulloa[14], The Genomics Education Partnership 1) Bronx Community College, Bronx, NY; 2) New Jersey City University, Jersey City, NJ; 3) Grand Valley State University, Allendale, MI; 4) Penn State Berks, Reading, PA; 5) Washington University in St. Louis, St. Louis, MO; 6) Rutgers University, Piscataway, NJ; 7) Moravian University, Bethlehem, PA; 8) Vassar College, Poughkeepsie, NY; 9) University of Northern Colorado, Greeley, CO; 10) Grinnell College, Grinnell, IA; 11) Columbia College, Columbia, SC; 12) University of Alabama, Tuscaloosa, AL; 13) Ohio Northern University, Ada, OH; 14) Lewis & Clark College, Portland, OR

The Genomics Education Partnership (GEP; thegep.org) is a nationwide collaboration of faculty from 180+ institutions with the goal of increasing understanding of genetics and familiarity with bioinformatics in diverse student populations through the incorporation of Course-based Undergraduate Research Experiences (CUREs) in the curriculum. Participating institutions range from community colleges, to PUIs, MSIs, HBCUs, and R1 universities. To facilitate both faculty and student access to curriculum materials and support active participation in the research, GEP provides a web-based platform with curated curriculum/training materials that can easily be incorporated into existing courses. During the COVID-19 pandemic, the accessible and immersive GEP curriculum and custom bioinformatics tools provide an inexpensive online framework for students to participate in research despite the lack of access to a traditional lab environment. During the 2020-2021 academic year, GEP reached over 3900 students. Through the GEP curriculum, students learn to annotate newly-sequenced eukaryotic genomes. They learn to leverage evidence from related informant species, experimental data (e.g., RNA-Seq), gene prediction algorithms, evolutionary conservation, and basic molecular biology rules to create a defendable gene model. The GEP research projects include investigation of venom evolution in parasitoid wasps, the evolution of insulin pathway genes across 27 Drosophila genomes, and expansion of the F element in four Drosophila species. We plan to expand the projects to additional species. Student gene models are reconciled and collated to generate a large dataset for evolutionary genomic studies, with student/faculty co-authors. In addition, GEP supports the publication of the gene models as microPublications, with students as lead authors. The GEP also engages in science education research. Our recent findings suggest that student perceptions of science, positive and negative, impact student learning outcomes. In addition to accessing the curriculum materials and tools, GEP faculty benefit from professional development opportunities and the support of a national network of like-minded colleagues. With the support of NSF and NIH, GEP is actively recruiting additional faculty members, particularly at MSIs and

community colleges, and both science and science education partners to collaborate on additional projects. Supported by NSF grants 1915544 and 1431407, and NIH R25GM130517.

1047B DrosoPHILA: a partnership between teachers and scientists that begins in the lab and continues in city schools *Kaitlin Laws*[1], Anthony Natale[2,3], Edward Waddell[1,4], Jamie Shuda[2,3], Greg Bashaw[1] 1) University of Pennsylvania Perelman School of Medicine; 2) University of Pennsylvania Institute for Regenerative Medicine; 3) University of Pennsylvania Netter Center for Community Partnerships; 4) Holy Family University

While exposure to laboratory activities often awakens student interest in science, many schools lack the resources to offer such experiences. Outreach sponsored by local colleges and universities can provide students with first-hand knowledge of the scientific process, reinforce learning objectives, and connect them to scientists in their community. Our program, DrosoPHILA, is a two-part high school curriculum built by teachers in the School District of Philadelphia (SDP) with input and support from members of the Bashaw lab and experienced outreach educators. In the first phase of this collaboration, three teachers from SDP conducted paid research in the Bashaw lab while helping to develop outreach modules. These modules, Flies on Ice and Roundabout We Go!, highlight the utility of *Drosophila* as a model organism for neurobiology research while reinforcing students' understanding of the scientific method and quantitative reasoning. In Flies on Ice, students determine how the time flies spend anesthetized on ice affects their recovery. In Roundabout We Go!, students study larval crawling in mutants with aberrant nervous system development. Each module is scaffolded by grade-appropriate presentations, student-driven experimentation, and guided data analysis. Our team leads at least two fifty-minute class periods of instruction.

In phase two of our program, we invited six SDP teachers to a two-day professional development program in the Bashaw lab, where they participated in our modules. During the 2018-2019 school year, our team led visits to these teachers' classrooms to guide students through both modules, reaching 252 students. In Fall 2019, we expanded our reach, visiting 14 classrooms (349 students). At the onset of the COVID-19 pandemic, we transitioned our program online and reached nearly 500 students by remote instruction. In Fall 2021, we resumed classroom visits, reaching more than 600 students across 21 classrooms. By surveying students before and after our visits, we were able to identify whether and how DrosoPHILA affected their knowledge and science identity. Our preliminary analysis indicates that our modules improve students' understanding of what it is like to be a scientist. Furthermore, because we surveyed students receiving both remote and in-person instruction, we are positioned to ascertain differences in outcomes under these circumstances. The teacher-informed design of our modules, along with the broad use of *Drosophila* as a model organism, makes them suitable for school districts across the United States.

1048C Investigating the impacts of engaging undergraduates as developers of inclusive curriculum through a service-learning course *Blake Riggs*[1], Maurina Aranda[2], Jeffrey Schinske[3], Laura Burrus[1], Kimberly Tanner[1] 1) San Francisco State University; 2) Southern Illinois University Edwardsville; 3) Foothill College

Scientist Spotlights – curricular materials that employ the personal and professional stories of scientists from diverse backgrounds – have previously been shown to positively influence undergraduate students' relatability to and perceptions of scientists. We hypothesized that engaging students in authoring Scientist Spotlights might produce curricular materials of similar impact, as well as provide a mechanism for student involvement as partners in science education reform. To test this idea and investigate the impact of student-authored Scientist Spotlights, we developed a service-learning course in which teams of biology students partnered with an instructor to develop and implement Scientist Spotlights in a biology course. Results revealed that exposure to 3 or 4 student-authored Scientist Spotlights significantly shifted peers' perceptions of scientists in all partner courses. Interestingly, student-authored Spotlights shifted peers' relatability to scientists similarly among both white students and students of color. Further, student authors themselves showed increases in their relatability to scientists. Finally, a department-wide survey demonstrated significant differences in students' perceptions of scientist representation between courses with and without student-authored Spotlights. Results suggest that engaging students as authors of inclusive curricular materials and partners in reform is a promising approach to promoting inclusion and addressing representation in science.

1049A Extending the Fly-CURE into an Upper-Level Undergraduate Bioinformatics Course *Kayla Bieser*[1], Richard Tillett[2], Jacob Kagey[3], Karen Bieluch[4], Nikolaos Tsotakos[5] 1) Nevada State College; 2) University of Nevada Las Vegas; 3) University of Detroit Mercy; 4) University of Maine; 5) Penn State Harrisburg

The Fly-CURE is a Course-based Undergraduate Research Experience (CURE) in which undergraduate genetics students from multiple US institutions characterize and conduct deficiency mapping of mutants in *Drosophila melanogaster*. A Flp/FRT EMS screen was conducted in the *Drosophila* eye in the context of blocked apoptosis to screen for conditional mutants that alter control of cell growth and development. The goal is to identify and map the mutation to a genomic region and/or gene on 2R. However, the gene and molecular location of some mutants have proven difficult to map utilizing these complementation techniques. To identify the mutations, we conducted whole genome sequencing (WGS) and bioinformatics analysis utilizing stocks of heterozygous EMS mutated flies with an unknown mutational locus, but

in some instances with a known genetic region mapped. A bioinformatics workflow was successfully established and verified utilizing four EMS mutants identified in the initial screen and a control stock representing their pre-EMS genetic background. When combined with deficiency mapping data, this methodology successfully identified mutated SNPs. To broaden participation and interest in bioinformatics, a scaffolded curriculum with the Fly-CURE was created at Nevada State College, a 4-year HSI. A 400-level bioinformatics course was implemented with a virtual format in Fall 2020 utilizing GitHub for course content and an app was developed in the CyVerse Discovery Environment for terminal and data access. A modification of Data Carpentries Genomics curriculum was utilized to introduce bash, the Unix command line and genomics methods, followed by a tailored hands-on pipeline for student analysis of WGS data collected from mutant flies the students had previously studied in the Fly-CURE. To-date, 49 students have completed the course and analyzed the genomes of 14 EMS mutant *Drosophila*, with eight mutations having been conclusively determined via this methodology. Preliminary assessment data supports that most students had little to no prior experience in bioinformatics and made significant gains in their understanding of file structures, Unix tools, Next Gen Sequencing, and their understanding of bioinformatics and how it relates to studying real world problems. The scaffolding of curriculum from genetics into bioinformatics allowed for greater understanding and buy-in by the students. Future goals include expansion of the curriculum to other institutions.

1050B Comparitive Effectiveness of Antioxiant and Lowered Carbohydrate Diets on Dysplastic Guts *Sandra Illescas*, Megan Hampton, Marie Page, Jessica Pacheco California State University Northridge

***Drosophila melanogaster* is a well-established model system within the biomedical research community, however, there currently exists a large gap in the field: the lack of a standardized diet. Standardization is critical for understanding the effects of dietary changes on stem cells in *Drosophila melanogaster*. This study sought to determine the effects that reducing carbohydrates and/or adding antioxidants to the diet could have on intestinal stem cells (ISCs) in the *Drosophila melanogaster* midgut. Our study analyzed morphological changes in ISCs that occurred as a result of the use of one of three dietary groups designed to work against oxidative stress in contrast with the control. By using four experimental groups, this study was able to consider the effects of reducing sources of oxidative stress, providing protective measures against oxidative stress, and the potential synergistic or antagonistic effects between these two dietary manipulations. Experimental groups had a reduction of carbohydrates, the inclusion of antioxidants, or the combination of both manipulations. This multimodal approach allowed the observation of morphological changes in the ISCs that could potentially only be a result of the overlap between the two variable changes. We hypothesized that a diet lower in oxidative stress could ameliorate premature dysplasia in the *Drosophila melanogaster* midguts. Intestinal samples were analyzed using whole mount fluorescent microscopy and CellProfiler to quantify changes in the number and/or morphology of ISCs between experimental groups. Data was collected using CellProfiler to measure the cell count and area of GFP stained cells, and normalized to DAPI stained cells. A one-way analysis of variance (ANOVA) with a 95% confidence interval showed a significant difference among groups (p=0.007), that could be attributed to the combination of lower carbohydrates and added antioxidants, as determined by a post hoc Tukey test. These initial results would indicate that the addition of antioxidants in combination with the reduction of carbohydrates appears to promote a higher density of progenitor cells in ISCs of Drosophila melanogaster, contrary to our original prediction. Our work demonstrates how even slight dietary manipulations can have detectable effects on ISC biology and will hopefully serve as a basis for future studies exploring the link between diet and intestinal homeostasis.**

1051C Students who participate in Fly-CURE demonstrate gains in self-efficacy and belonging across a Research Coordination Network both before and during the COVID-19 pandemic *Jacob Kagey*[1], Kayla Bieser[2], Joyce Stamm[3], Alysia Vrailas-Mortimer[4], Fly-CURE 1) University of Detroit Mercy, Detroit, Michigan; 2) Nevada State College, Las Vegas, Nevada; 3) University of Evansville, Evansville, Indiana; 4) Illinois State University, Bloomington, Illinois

Fly-CURE is a consortium of fifteen institutions implementing a *Drosophila* centered research project within undergraduate genetics laboratory courses. In this CURE, students work to characterize and map novel EMS mutants isolated from a Flp/FRT screen. To date, students have mapped 19 mutants on chromosome 2R including alleles of *Egfr, Shot, Hpo,* and *Shn.* These findings have resulted in six micropublications with 368 student co-authors. To understand the impact of Fly-CURE on student learning and attitudes towards research, we have assessed students on their perceived changes in sense of self-efficacy and sense of belonging, as well as gains on genetics-based learning objectives. These results were segregated by whether students had a previous research experience prior to the Fly-CURE. Overall, students who participated in the Fly-CURE report statistically significant gains in science self-efficacy and sense of belonging. Further, those students without a prior research experience reported lower scores in science self-efficacy before the class but have levels of science self-efficacy indistinguishable from those with previous research experiences by the end of the Fly-CURE. During the academic transition resulting from the COVID-19 pandemic, Fly-CURE labs were taught using virtual, hybrid, and in-person modalities. Despite these changes in delivery methods, we see no statistical differences in student-reported gains before or during the pandemic. The Fly-CURE is an adaptable *Drosophila* based CURE that impacts student attitudes towards research and contribute to the understanding of the genetic regulation of developmental growth.

1052A A Research-based laboratory course in Molecular Biology, Genetics, and Evolution Eric Spana Duke University

A introductory level course at Duke University in Molecular Biology, Genetics, and Evolution required a lab component that spanned as much of these three subjects as possible. I have developed a lab that uses local, wild-caught fruit fly species (mostly *Drosophila*) as the starting point for a laboratory portion in the course. Students choose flies from the collection and use PCR and sequencing of the Cytochrome Oxidase I gene to identify the species via DNA barcoding analysis. After phylogenetic analysis of the collected species, students amplify a region of a second gene, the Tyrosine Hydroxylase gene (TH) using degenerate PCR primers. This PCR product is then cloned into a TA cloning vector. After transformation, mini-preps, and restriction digest analysis, students can identify which samples contain the appropriate cloned DNA. Sequencing and analysis of the plasmid insertions follows and a number of evolutionary analyses are performed on the sequence. The region of TH that is being sequenced covers both introns and exons, and there is a cis-regulatory element in one intron that drives expression in the larval brain in *D. melanogaster*. Alignment of the TH region sequences identified a few highly conserved regions within the intron that contains the cis-regulatory element. I have run this course at two different Universities so far: Duke University and Duke Kunshan University, and have identified ~22 species in Durham, NC and ~11 in Kunshan, China with an overlap of only three species and at least one unidentified species in each location. This laboratory gives students experience in many common molecular biology techniques: PCR, cloning, plasmid DNA isolation, restriction digests, agarose gels, and sequencing as well as analysis of their own data for gene structure, reading frames, codon usage, dN/dS ratio, MK test, phylogeny. An online version of this lab used extensive BLAST and gene annotation from 80+ sequenced genomes to arrive at a similar result.

1053V Reproducibility for Everyone April Clyburne-Sherin[1], *Nele Haelterman*[2], Ruchika Bajaj[3], Reproducibility for Everyone Group 1) Reproducibility for Everyone, Brooklyn, NY; 2) The Scientist, Wilmington, DE; 3) UCSF, San Francisco, CA

Rigor and reproducibility are at the core of modern science. Many new initiatives and tools have been established to address barriers to reproducibility. While very welcome, these projects have led to a proliferation of online tools and resources which can be hard to sift through.

Reproducibility for Everyone (R4E) is a global, community-led reproducibility education initiative. R4E runs practical and accessible workshops to introduce the concept of reproducibility to researchers. We demonstrate reproducible tools and methods that can improve research by making it more efficient, transparent, and rigorous. Every R4E workshop is customized for the audience. For *Drosophila* 2022, R4E instructors will focus on aspects of reproducibility most relevant to *Drosophila* research and share resources and tools curated for the GSA community.

This workshop will introduce reproducible workflows and a range of tools along the themes of organization, documentation, analysis, and dissemination. After a brief introduction to the topic of reproducibility, the workshop will provide specific tips and tools useful in improving daily research workflows. The content will include modules such as data management, electronic lab notebooks, reproducible bioinformatics tools and methods, protocol and reagent sharing, data visualization, and version control. All modules include interactive learning, real-time participation, and active knowledge sharing. The methods and tools introduced help researchers share work with their future self, their immediate colleagues, and the wider scientific community.

Following this workshop, participants will be able to:
1. Apply a conceptual framework for reproducibility, replicability, and robustness of research.
2. Explore practical, accessible tools and methods for advancing the reproducibility of research.
3. Reuse and adapt the Reproducibility for Everyone modular curriculum to their own training and research needs.
4. Evaluate their reproducibility barriers and solutions through interactive knowledge sharing.

1054V A semester-long genetics lab exploring gene families through comparative genomics and CRISPR-based gene editing Jennifer Kennell Vassar College

Course-based undergraduate research experiences (CUREs) offer students opportunities to gain the benefits of conducting independent research in the context of a course laboratory. Recently my research lab has become interested in studying members of the Haloacid Dehalogenase (HAD) family of non-protein phosphatases. In particular, we began by characterizing in *Drosophila* possible orthologs of Phosphoglycolate Phosphatase (PGP), an enzyme that was recently identified as playing an evolutionarily conserved role in dephosphorylating toxic side products of glycolysis. Using the tools I have learned through participation in the Genomics Education Partnership (GEP) Pathways Project, I have developed a CURE for my intermediate-level Molecular Genetics class that involves annotation of possible orthologs of these phosphatases in various *Drosophila* species. Each semester students annotate a different family member and perform evolutionary analyses of the predicted gene and protein sequences. In addition, they also generate a mutation in the *D. melanogaster* ortholog using transgene based CRISPR-Cas9 gene editing; these mutant lines can then be used in future studies by independent research students in my laboratory. Through the gene annotation portion of the project

students get hands on experience with comparative genomics approaches while reinforcing their understanding of eukaryotic gene structure and evolution. And through the mutagenesis portion of the project they gain experience in setting up fly crosses, analyzing progeny, and conducting molecular analyses to identify possible mutations in the new fly lines they generate. The guide RNA transgenic lines necessary for the project are readily available through various stock centers or are fairly straightforward to generate and make excellent independent projects for undergraduates. This CURE is very flexible and can easily accommodate any gene or gene family of interest. The mutagenesis portion of the project can also easily be incorporated into the existing GEP Pathways Project studying the Insulin pathway.