66th Annual *Drosophila* Research Conference

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Abstract Book

GSA GENETICS G3

1 **RACing from Drosophila border cell migration to an enhanced cellular immunotherapy** Denise Montell University of California Santa Barbara

RACing from Drosophila border cell migration to an enhanced cellular immunotherapy

2 **Basement Membrane Repair** Andrea Page-McCaw Vanderbilt University

Drosophila has long been a pioneer in analyzing cells and tissues in vivo, and more recently flies are leading the way in understanding how cells and tissues interact with extracellular matrix. Like nearly all animals, flies have a conserved flat extracellular matrix known as basement membrane that underlies epithelia and wraps around muscles, nerves, tracheae, and fat. In vitro studies of basement membrane proteins have been highly successful in illuminating its structure, but the dynamics of basement membranes have been largely overlooked until recently, thanks in large part to work in flies. My lab studies tissue repair after injury. We have focused on the repair of basement membranes, about which virtually nothing was known, addressing questions such as how is damage is detected, what cells repair the damage, and how is repair controlled to avoid fibrosis?

The most abundant structural protein of basement membranes is Collagen IV (Col4), made of the components Viking (Vkg) and Cg25C. Using the intestinal basement membrane of adult Drosophila as a model, we found that after basement membrane damage, there is a sharp increase in enteroblasts transiently expressing Col4, and we call these “matrix mender” cells. Enteroblast-derived Vkg and Cg25C are specifically required for matrix repair. The damage-induced increase in matrix mender cell number requires the mechanosensitive ion channel Piezo, expressed in intestinal stem cells. Matrix menders arise in response to the loss of matrix stiffness that accompanies damage, as specifically inhibiting Col4 crosslinking is sufficient for Piezo-dependent induction of matrix mender cells. Our data suggest a general model that epithelial stem cells control basement membrane integrity by monitoring its stiffness.

3 **Investigating Endoplasmic Reticulum Dynamics in Asymmetric Cell Division and Cell Fate Determination** Blake Riggs San Francisco State University

Cell division is a fundamental process in multicellular life, with extensive research focused on the precise segregation of genetic material into daughter cells. However, the mechanisms governing the partitioning and inheritance of cytoplasmic components and organelles remain less understood. Our research explores the reorganization, monitoring, and inheritance of organelles during cell division, with a particular focus on the endoplasmic reticulum (ER).

The ER, the largest cellular organelle, plays critical roles in protein synthesis, modification, and calcium homeostasis. Through our studies, we have demonstrated that mitotic ER reorganization is coordinated by the astral microtubule network and centrosomes, aligning dynamically with the cell cycle. Recent efforts have uncovered the ER’s role in generating cell diversity through the asymmetric partitioning of cell fate determinants.

During Drosophila gastrulation, we observed asymmetric ER partitioning in proneuronal cell divisions. This process is mediated by the conserved ER transmembrane protein Jagunal (Jagn). Loss of Jagn function disrupts ER asymmetry, leading to symmetrical ER distribution and a supernumerary neuroblast phenotype. Furthermore, Jagn inhibition results in the mislocalization of key cell fate determinants, including the apical Par protein complex, crucial for cell polarity, and the basal transcription factor Prospero (Pros).

Our findings reveal that Jagn regulates intrinsic signals driving cell fate decisions during asymmetric division by mediating the transport and localization of cell fate determinants. These results highlight a novel role for the ER in cell differentiation and provide broader insights into the mechanisms of cell division, with implications for understanding genetic disorders, tumorigenesis, and neurodegenerative diseases.

4 **Microbial Influence on Drosophila Adaptive Growth** François Leulier IGFL CNRS-ENS de Lyon

Animals establish reciprocal interactions with their commensal bacterial communities. Despite recent progress, a clear view of the physiological benefits associated with host-microbiota relationship remains elusive. Hence the molecular mechanisms through which the microbiota exerts its beneficial influences on animal biology are still largely undefined. My research team aims at deciphering the molecular dialogue governing the mutualistic interaction between intestinal bacteria and their animal host. To this end, we are using a genetically tractable gnotobiotic insect model: *Drosophila melanogaster,* associated to its natural dominant commensals, *Lactiplantibacillus plantarum* and *Acetobacter pomorum*. We are developing multiscale functional approaches to identify the mechanisms that underlie their mutualistic relationship, which results in the support of host adaptive growth upon nutritional challenges, especially poor nutritional conditions. Our approaches aim at identifying both the bacterial and host genetic and metabolic networks required to sustain their mutualistic relationship.

5 **Chemical Diversification in *Drosophila*: Insights from Genetics, Microbiota, and Biogeography** Joanne Y. Yew Pacific Biosciences Research Center, University of Hawaii at Manoa

Chemical signals are the language by which flies communicate with each other and engage in inter-kingdom interactions with microbes and plants. Understanding how chemical communication systems originate, diversify and drive evolution is a central question in sensory biology. In this talk, I will explore the genetic, microbial, and environmental factors that shape the diversification of lipid pheromones and metabolites. We use a combination of *Drosophila* genetic tools and mass spectrometry to elucidate molecular mechanisms that underlie chemical diversification. Our past work has shown that the novel expression pattern of a single lipid biosynthetic enzyme in a pheromone gland changes the chemical profile of the gland and results in the emergence of a new pheromone. Beyond genetics, we have found that the microbial community of bacteria and fungi associated with flies contribute to chemical plasticity by altering lipid metabolism, steroid levels, and life history strategies. Currently, we are integrating the well-described biogeography of the Hawaiian *Drosophila* radiation with chemical and microbial characterization in order to gain a macroscopic perspective on the roles of abiotic and microbial factors in the evolution of chemical signals across space and time.

6 **Evolution of Morphogenesis** Pavel Tomancak Max Planck Institute of Molecular Cell Biology and Genetics

My laboratory is interested in the evolution of morphogenesis on a broad macroevolutionary scale. To that end, we use multiple model and non-model species to compare the cellular and molecular mechanisms of equivalent morphogenetic events across the animal phylogeny. Drosophila remains the premium system for addressing the interplay between gene regulation and tissue mechanics from the mechanistic perspective. In Dresden, we have pioneered biophysical techniques and computational modeling to understand tissue behavior on the mesoscale. I strongly believe the systematic application of these quantitative approaches in the comparative context will bring further progress in developmental biology. To apply the biophysical toolkit in various model systems, my laboratory has, over the years, developed several broadly applicable open-access tools for imaging (OpenSPIM) and image analysis (Fiji platform and its plugins). I will discuss the latest developments in large-scale analysis of developmental lineages using the powerful Mastodon software (not to be confused with the social network). On the biology side, I will show how we use these tools and the general interdisciplinary approach to understand the developmental function and evolutionary origin of early gastrulation processes in Drosophila and beyond.

7 **Cell cycle variations in development and cancer** Brian R Calvi[1], Yi-Ting Huang[2], Hunter Herriage[2] [1]Biology, Indiana Univ, [2]Biology, Indiana University

Cell cycle variations are common in development and contribute to disease. One common variant is the endocycle. During endocycles cells grow and periodically duplicate their genome without dividing, which results in large, polyploid cells. Endocycles are a normal growth program of specific tissues in a wide variety of organisms. Cells can also switch to endocycles in response to conditional signals. We call these induced endocycling cells (iECs), or unscheduled endocycles, to distinguish them from the programmed developmental endocycling cells (devECs) that occur during normal tissue growth. iECs can be beneficial for wound healing, but also contribute to cancer. It is underexplored what determines these disparate effects of iECs and how similar their cycling and growth are to that of endocycles in development. We showed how remodeling of the cell cycle transcriptome promotes a switch to endocycles in both iECs and devECs, and that this switch represses the apoptotic response to DNA damage. Unlike most devECs, however, we found that *Drosophila* and human iECs were able to resume divisions that generate aneuploid daughters. Current evidence now supports that unscheduled endocycles contribute to cancer therapy resistance and, upon return to division, cancer progression.

We have continued to use *Drosophila* to define the impact of unscheduled endocycles on cell, tissue, and tumor growth. Induction of premature ovarian follicle cell endocycles caused pleiotropic defects in oogenesis and perturbed epithelial form and function. In the wing disc, iECs initially grew in cell size, but then underwent a Jun N-terminal kinase (JNK) mediated senescent arrest that caused tissue undergrowth. Unlike other growth-compromised wing disc cells, iECs were naturally apoptosis-resistant and not eliminated from the epithelium. These persistent, senescent iECs promoted division of diploid neighbors, but this compensatory proliferation failed to rescue tissue growth, and instead caused aberrant overgrowth. Surprisingly, iEC senescence was reversible with some undergoing error-prone, polyploid mitotic divisions. Our recent results are revealing pathways that are activating JNK in growth-compromised iECs and the relationship to wound healing and tumorigenesis. We have also found that oncogenic mutations synergize with endocycles to promote tumorigenesis. I will discuss the broader implications of these findings to understanding the contribution of polyploid cells to human cancer.

8 **DrosoPHILA: a model for sustainable outreach programming** Kaitlin Laws[1], Ent Natale[2], Edward Waddell[3], Jamie Shuda[4], Greg J Bashaw[4] [1]Biology, Randolph-Macon College, [2]Rowan University, [3]Holy Family University, [4]University of Pennsylvania

Outreach programming creates connections between scientists and their communities while expanding students' perception of what science entails and who practices it. This programming can act as one part of a multipronged approach to diversify the scientific workforce. To build sustainable, effective science outreach curricula, scientists should seek input from both teachers in their communities and experienced outreach educators. Our outreach program, DrosoPHILA, can serve as a model for such partnerships. DrosoPHILA leverages the tools of the Bashaw lab's fly neurodevelopmental research program at the University of Pennsylvania to reinforce the biology curriculum in local public schools. DrosoPHILA was developed and is sustained by a continued collaboration between current and former members of the Bashaw lab, experienced outreach educators, and teachers in the School District of Philadelphia. By explaining the program's development and making our supporting materials available, we hope to facilitate the creation of similar programs across different subject areas.

9 **Meeting the Moment: Using Conversations to Bridge Divides and Build Mutual Understandings** Marnie E. Gelbart Department of Genetics, Harvard Medical School

As a genetics community, what we do today will profoundly shape the legacy of our science. Advances in the field are rapidly outpacing the speed with which people can learn about, understand and grapple with their far-reaching applications and implications. Unfortunately, discussions of such issues are often siloed within professional fields or communities–with some communities excluded entirely. With growing social inequities and polarization on vaccines, climate, race, gender, and other topics, many researchers and scientific societies have recognized the need for scientists to participate in and expand efforts to connect science and society. In our 19 years of public engagement experience, the Personal Genetics Education & Dialogue (PGED) team has seen the impacts of inclusive conversation on building rapport, growing mutual understandings, empowering individuals in decision-making, and enriching the processes and directions of science. In this talk, I will highlight PGED's free resources and share experiences engaging with high school and university students and educators, library patrons, community health advocates, scientists, faith leaders from diverse religious traditions, congressional staffers on both sides of the aisle, and storytellers, among others. In addition, I will introduce a new collaboration with the Genetics Society of America (GSA) and the Reclaiming STEM Institute, where we bring what we have learned from public engagement to issues of culture change within the genetics field.

10

Dodging replication forks to express genes during S phase Chun-Yi Cho[1], Patrick H O'Farrell[2] [1]Biochemistry, UCSF, [2]Biochemistry, Univ California

Live imaging of embryos shows pattern and structure emerging in a smooth and orderly manner. This involves the coordination of many processes, each of which needs to be precisely regulated in time. We have been studying aspects of temporal control, especially of cell cycle events, in the early embryo, and recently have addressed one of the most fundamental problems of timing coordination. Genes are transcribed throughout S phase, yet the DNA template cannot be used for both replication and transcription at the same time. We argue that temporal control within S phase is widely important in biology to avoid collisions, and that the early *Drosophila* embryo offers a unique opportunity to determine how effective temporal control operates. The early wave of transcription in the *Drosophila* embryo occurs during the rapid nuclear division cycles in which the entirety of interphase is occupied by an exceedingly rapid S phase. Because transcript elongation requires much of the time of early interphases, transcription initiating on unreplicated sequences will inevitably be confronted with invading replication forks resulting in failure of transcription and DNA damage. Hence, we hypothesized that all transcription during these stages initiates on replicated DNA. Simple mechanisms that delay the onset of transcription could coordinate the processes so that transcribing RNA Pol2 can follow replication forks without interference. We will present findings showing that onset of transcription in each cell cycle is delayed so that it follows replication, which is directed by a bookmarking process to initiate replication of transcribed genes especially early. We will discuss what is needed to extend the findings in this specialized biological setting to a general solution that would avoid conflicts between replication and transcription throughout eukaryotic biology.

11 **Extracellular matrix restrains cell cycle progression by nuclear exclusion of Yorkie in *Drosophila*** Liyuan Sui[1,2], Elisabeth Fischer-Friedrich[2], Christian Dahmann[1,2] [1]School of Science, Technische Universität Dresden, [2]Cluster of Excellence Physics of Life, Technische Universität Dresden

Tissues and organs grow to a characteristic final size during animal development. A hallmark of tissues reaching their final size is the cessation of cell division. Among the multiple mechanisms that have been proposed to explain the cessation of cell division, mechanical compression of the tissue recently received particular attention. However, how mechanical compression is imparted on tissues and how mechanical compression results in cessation of cell division is not fully understood. In *Drosophila* late larval wing discs, cell cycle progression slows down and cells ultimately arrest in G2 phase. Here, we show that depletion of the extracellular matrix (ECM) in late larval wing discs decreases tissue compression, releases cells from G2 phase arrest and promotes cell division. Moreover, we show that ECM depletion in late larval wing discs results in the nuclear accumulation of the growth-promoting transcriptional co-factor Yorkie, which is normally excluded from the cell nucleus at this larval stage. Finally, we show that nuclear exclusion of Yorkie plays a critical role in mediating cell cycle cessation in response to ECM-mediated mechanical tissue compression. Our work indicates that mechanical compression imparted on the tissue by the ECM plays an important role in determining final tissues size. Moreover, our work also demonstrates that ECM restrains cell cycle progression, and thereby contributes to final tissue size, by nuclear exclusion of Yorkie.

12 **Exploring the effects of muscle-generated mechanical constraints acting on *Drosophila* midgut tissue** Benedicte M. Lefevre, Nicky Efstathiou, Allison J Bardin Team Stem Cells and Tissue Homeostasis, Institut Curie, CNRS, UMR3215, INSERM U934, PSL Research University

Cells and tissues undergo changing mechanical constraints during development, due to physiological activities of organs, or during tumorigenesis. The effects of these constraints have raised more and more interests in the last decade and have been mostly studied in developmental or pathological context. However, these constraints and their consequences on cells have received far less attention on complex, adult tissues. Due to its physiology, the *Drosophila* midgut tissue undergoes variations of mechanical constraints *in vivo*, due to food transit and muscle activity. Here, we use this well-characterized tissue to investigate how its cells, enterocytes, enteroendocrine cells, and in particular stem cells, cope with varying mechanical constraints. To alter the mechanical environment of the midgut, we perturbed the contractility of its surrounding muscles, increasing or decreasing it. We found out that muscle contractility is crucial for maintaining organ size and shape and has important roles at tissue- and cellular- scales. In particular, reduction of muscle contractility triggers rapid organ enlargement, tissue flattening, and abnormal stem cell mitoses. Combining imaging of fixed and live samples, as well as RNAseq, we are characterizing this phenotype to unravel how changes to the surround muscle impact mechanical alteration of the epithelium environment to trigger changes in organ size, shape and stem cell mitoses. I will present the ongoing results of this project. In a broader perspective, we plan to investigate cell-type specific biomechanical properties and thus potential differential sensitivities to mechanical constraints. Altogether, this work will help define adult intestinal responses to mechanical changes and has broader implications for our general understanding of epithelial biology and tissue-tissue biomechanical interactions.

13 **Unscheduled endocycling and oncogenic mutations synergize to promote cell growth and tumorigenesis.** Hunter C Herriage[1,2], Sarah K Fahey[3], Brian R Calvi[1,2] [1]Biology, Indiana University Bloomington, [2]Indiana University Melvin and Bren Simon Cancer Center, [3]Indiana University Bloomington

The endocycle is a variant cell cycle characterized by oscillating G/S phases without an intervening cell division, resulting in large, polyploid cells. Endocycling cells are important for the normal development of many eukaryotes. However, recent data indicate that *unscheduled* endocycling can be detrimental to tissue growth and function, with one example of significance being Polyploid Giant Cancer Cells (PGCCs). PGCCs have recently become appreciated as a biomarker for poor prognosis in cancer patients. However, much remains unknown about the control of PGCC growth and proliferation and how they affect tumor growth. In this presentation, I will present my work using *Drosophila* to investigate the synergies between unscheduled endocycles and oncogenic mutations as a model for PGCCs.

We used the GAL4/UAS system to switch wing imaginal disc cells into the endocycle, resulting in cells which grew to large size (hypertrophy), then underwent a senescence-like arrest (Huang *et al.*, 2024, PLOS Genetics, 20(9), e1011387). We call these induced endocycling cells (iECs). We found that iECs expressing the conserved Ras^{V12} or *yorkie* (*yki*) oncogenes grew larger and more polyploid than iECs alone. Cell cycle analysis indicated that Ras^{V12} expression overcame the senescent-like arrest of iECs. However, overall growth of the tumor was not demonstrably increased when compared to tumors expressing Ras^{V12} or *yki* alone. Therefore, although oncogene expression promotes iEC cell cycle progression and hypertrophy, iECs do not enhance overall tumor growth. Evidence suggests that PGCCs can return to mitotic divisions which promote tumor progression, but how oncogenic mutations affect these cell division dynamics is unknown. To address this question, I generated a new *LexAop-*Ras^{V12} fly strain that permits constant Ras^{V12} expression while inducing *transient* endocycles that can return to division using the GAL4/$GAL80^{ts}$ system. Strikingly, Ras^{V12} expression promoted the survival and proliferation of polyploid iEC daughter cells with hallmarks of genome instability. This return to error-prone divisions resulted in the growth of large tumors. Thus, *transient* endocycling and oncogenic mutations synergize to promote tumorigenesis and genome instability. I will report on our continued analysis of this synergy between oncogenic mutations and iECs, and I will discuss the broader impact of this work on our understanding of PGCCs in cancer.

14 **Mechanisms of PP2A-Ankle2 dependent nuclear reassembly after mitosis** Jingjing Li[1], Xinyue Wang[1], Laia Jordana[1], Éric Bonneil[1], Victoria Ginestet[1], Momina Ahmed[1], Mohammed Bourouh[1], Cristina Mirela Pascariu[1], T. Martin Schmeing[2], Pierre Thibault[1], Vincent Archambault[1] [1]IRIC - Université de Montréal, [2]Biochemistry, McGill University

In animals, mitosis involves the breakdown of the nucleus. The reassembly of a nucleus after mitosis requires the reformation of the nuclear envelope around a single mass of chromosomes. This process requires Ankle2 which interacts with PP2A and promotes the function of Barrier-to-Autointegration Factor (BAF). Upon dephosphorylation, BAF dimers cross-bridge chromosomes and bind lamins and transmembrane proteins of the reassembling nuclear envelope. How Ankle2 functions in mitosis is incompletely understood. Using a combination of approaches in *Drosophila*, along with structural modeling, we show for the first time that Ankle2 is a regulatory subunit of PP2A, explaining how it promotes BAF dephosphorylation. In addition, we discovered that Ankle2 interacts with the endoplasmic reticulum protein Vap33, which is required for Ankle2 localization at the reassembling nuclear envelope during telophase. We identified the interaction sites of PP2A and Vap33 on Ankle2. Through genetic rescue experiments, we show that the Ankle2/PP2A interaction is essential for the function of Ankle2 in nuclear reassembly and that the Ankle2/Vap33 interaction also promotes this process. Our study sheds light on the molecular mechanisms of post-mitotic nuclear reassembly and suggests that the endoplasmic reticulum is not merely a source of membranes in the process, but also provides localized enzymatic activity.

15 **Studying Double-Stranded DNA Gap Repair Mechanisms Utilizing APOBEC3A Mutagenic Activity** Mohamed A Mahmoud[1], Josh Ponder[2], Jeff Sekelsky[2] [1]Department of Genetics, The University of North Carolina at Chapel Hill, [2]Department of Biology, The University of North Carolina at Chapel Hill

Revealing dsDNA gap repair mechanisms is essential for understanding genome stability and optimizing biological applications like CRISPR/Cas9. Error-free homologous recombination (HR) is responsible for faithful repair of DSBs and dsDNA gaps. Gaps, being several kilobase pairs in size, present a challenge for the repair machinery, differing from simple DSBs. Literature indicates that repair efficiency diminishes with increasing gap size, though the threshold for this decline remains unclear. The dependency of resection length, strand invasion, and DNA synthesis on gap size requires further exploration. D-loop dynamics during the HR DNA synthesis step is another poorly understood aspect of gap repair. The extended D-loop and migrating bubble models have been proposed to explain D-loop dynamics, with in-vitro evidence supporting the latter while in-vivo data is lacking. We intend to investigate the gap size-dependent efficiency of repair, propose a comprehensive model delineating D-loop progression dynamics, and examine the continuous resection associated with varying gap sizes. We employ two experimental assays to bridge the existing knowledge gap. The first assay, designated the p{wa} assay, induces a gap through transposable element excision via an inefficient transposase that cleaves only one sister chromatid. The uncut sister chromatid can subsequently serve as a template for repair. In the second assay, termed the ebony gap repair assay, we utilize the ebony locus on one chromosome as a target sequence for Cas9-mediated cleavage, wherein the other homolog, characterized by different sizes of insertions representing various gap sizes, can function as a template. Both assays incorporate APOBEC3A-mediated mutagenesis to ascertain the localization of ssDNA during the repair process. Through long-read sequencing, we analyze the target and template sequences to identify APOBEC3A-induced mutations and their patterns, which may elucidate the aforementioned scientific inquiries. Sequencing outcomes from the p{wa} assay have revealed a mutational signature indicative of a migrating bubble with synchronous invasion occurring at both termini of the gap. These findings may alternatively be interpreted as a transition from an extended D-loop to a migrating bubble. Given that the template chromosome cannot be retrieved in this assay, the results from the p{wa} assay can solely suggest a migrating bubble component of D-loop progression, while failing to provide a comprehensive depiction of D-loop dynamics. Consequently, the results from the ebony gap repair assay, wherein both the target and template chromosomes are recoverable, are considerably more definitive and yield a thorough characterization of D-loop progression dynamics.

16 **Calcium in Neural Development: Cell Cycle and Cell Fate** Jillian Wynne, Bernice C Lin, Isabella R Maag, Hannah Christman, Asher Swan Adams, Beverly Piggott Division of Biological Sciences, University of Montana

Within neural development, Ca^{2+} has been studied and implicated in most processes, including proliferation, migration, differentiation, and cell signaling. The role of Ca^{2+} in neural stem cell function and cell fate decisions is not as well understood. Our research investigates how Ca^{2+} dynamics affect asymmetric cell division (ACD) and how the role of Ca^{2+} changes based on cell identity. Drosophila melanogaster is a useful model for ACD. The distinct types of neuroblasts (NB - fly neural stem cells) vary in their patterns of division and marker expression. Preliminary results of our investigation showed that disrupting cytosolic Ca^{2+} levels through RNAi knockdowns of Ca^{2+} regulators reduced the number of cells in T2NB lineages in 96h ALH larvae, with Sarcoendoplasmic reticulum Ca2+ ATPase (SERCA) RNAi having the most severe effect on lineage size and causing a reduction in overall brain volume. Furthermore, a knockdown of SERCA in T2NB lineages had the unique effect of changing the T2NB lineages to a T1NB lineage identity. To investigate how the role of Ca^{2+} differs in regulating identity and proliferation, we knocked down cell cycle regulators to see if the SERCA knockdown effects are attributable to a cell cycle defect. Knocking down the cytokinesis regulator *pavorotti* did not affect T2NB identity, but when we knocked down *cyclin dependent kinase 1*, the T2NB identity transformed to a T1NB identity, suggesting that T2NB identity requires cell cycle progression but not cytokinesis. Since our data show that Ca^{2+} regulation is required for cell fate decisions, we also investigated how the role of SERCA differs between cell types. In T2NB lineages, an overexpression of SERCA was found to increase the number of cells in the lineages. However, when SERCA was overexpressed in all brain cells, the total brain volume was significantly reduced, suggesting that SERCA, and by extension, intracellular Ca^{2+} regulation, has a different function in T2NBs than in other cells. This research and our continued exploration of how SERCA is related to cell cycle regulation, cell fate decisions, and proliferation and how its effects differ in T1 and T2 NBs provides insight into the undefined mechanisms of Ca^{2+} regulation in neural development.

17 **Dual contributions of Xrp1 to genome integrity through the DNA damage response and cell competition** Chaitali Khan[1,2], Nicholas E Baker[3,4] [1]Cell and Developmental Biology Centre, National Institutes of Health, [2]Dept. Genetics, Albert Einstein College of Medicine, [3]Microbiology and Molecular Genetics, University of California, Irvine, [4]Dept. of Genetics, Albert Einstein College of Medicine

The tumor suppressor gene *p53*, known for its role in maintaining genomic integrity after DNA damage, has also been implicated in cell competition in mammals. The main transcriptional target of p53 in the Drosophila DNA damage response (DDR) is the bZIP domain transcription factor Xrp1, which we and others have shown is also responsible for the cell competition of Minute cells and other kinds of abnormal cell. We have therefore investigated whether Xrp1 is required to maintain genome integrity as a p53-effector in the DDR, or by eliminating damaged cells through cell competition later. Xrp1 mRNA and protein were induced in imaginal discs within 30 mins to 4 hours post irradiation in a p53-dependent manner, and were responsible for expression of the majority of " *p53* target" genes examined, which includes genes with functions in DNA repair, cell death and cellular stress signaling pathways, many of which contain binding motifs for Xrp1 but lack apparent binding sites for p53. This role of Xrp1 as an immediate early gene mediating the p53-dependent DDR was functionally significant because both DNA-damage induced apoptosis and the repair of double-strand breaks were incomplete in the absence of Xrp1, when only a subset of " *p53* target" genes are expressed. We confirmed that Xrp1 expression is activated again in a subset of cells ~24h post-irradiation, after the DDR. This later Xrp1 activity, which was independent of p53 and depended instead on the ribosomal protein RpS12 and on Xrp1 auto-regulation, eliminated cells that are thought to have become genetically Minute due to aneuploid and segmentally-aneuploid genomic changes that affect Ribosomal protein gene dose. Thus, Xrp1 makes dual contributions to genome integrity following DNA damage, first as an intermediate transcription factor mediating much of the transcriptional DDR downstream of p53, and secondly by competitively removing cells where DNA repair has failed or has changed the genotype. In mammals, where clear homologs of Xrp1 are difficult to identify, p53 may also act through intermediate transcription factors, and is also important in cell competition. It will be interesting to determine how cell competition contributes to the tumor suppression function of mammalian p53, which appears to be separable from the mammalian DNA Damage response.

18 **The evolution of *Drosophila's* innate immune responses to bacteria** Cong Li, Li Zhao The Rockefeller University

Immunity against diseases and pathogens is fundamental to the survival and health of living organisms. Research on innate immunity in fruit flies is primarily on how the model fruit fly species, *Drosophila melanogaster,* deploys its immune responses against bacteria, fungi, and wasps, leading to incomplete knowledge on the evolution of immune related genes in the fly phylogenetic tree, including those non-melanogaster species that are ecologically and evolutionarily important. Using both closely and distantly related non-model species to *Drosophila melanogaster* offers us the opportunity to study species or lineage-specific genes, which can often indicate the rapid evolution or innovation of genes that have essential functions. In this study, we focused on the various physiological reactions of five different fly species in response to bacterial infections by manual needle pricking. We examined if any novel genetic and functional components in the fly innate immune system were used against the same pathogenic challenge. We found significant differences in survival performance upon bacterial infection in the different fly species. We then linked this variance in survival to bacterial load inside the fly's body and found a positive correlation, suggesting that different fly species have distinct innate abilities to clear the pathogens. To have a comprehensive view of gene expression patterns in these flies upon bacterial infection, we generated RNA sequencing data for flies in five species and in three conditions: untreated, sterile-wound, and bacteria-infected. Using a comparative genomics study, we identified several lineage-specific genes that potentially encode antimicrobial peptides, a category of functional effectors in the insect immune system that are utilized to kill microbial pathogens. We then tested their antimicrobial properties using in vitro functional experiments with a minimum inhibitory concentration assay. Finally, we used a machine learning approach to predict and identify whether a gene tends to be an antimicrobial peptide-encoding gene or not. This study gives us a critical insight into how evolutionary novel and rapidly evolving genes can have important functions in the fly immune system.

19 **Feeding promotes the environmental transmission of an insect endosymbiont** Dylan Shropshire, Alphaxand Njogu, Helene Hartman, Callum Shutack Department of Biological Sciences, Lehigh University

Wolbachia is the most prevalent endosymbiotic bacterium, inhabiting the cells of over 50% of insect species worldwide, including *Drosophila melanogaster* and its relatives. As it is increasingly deployed across multiple continents to combat mosquito-borne diseases like dengue and Zika, understanding *Wolbachia*'s transmission dynamics has become critically important. While it is primarily transmitted vertically from mother to offspring, growing evidence indicates that *Wolbachia* can also spread horizontally between species. However, the mechanisms and conditions that enable such host switching are still poorly understood. A crucial step in this process likely involves the release of *Wolbachia* from host cells into the environment. We hypothesize that food may act as a conduit for *Wolbachia*'s environmental transmission. I present findings from a series of experiments that support this hypothesis, revealing key dynamics of *Wolbachia*'s environmental transmission and offering new insights into its widespread distribution and potential interactions with new hosts.

20 **Taste regulation of immunity** Pierre-Yves Musso[1], Alix Najera Mazariegos[2], Gérard Manière[3], Martine Berthelot-Grosjean[3], Darius Camp[2], Romane Milleville[4], George Alves[3], Yaël Grosjean[3], Julien Royet[4], Guy Tanentzapf[2] [1]TPI lab, CNRS, CSGA, [2]University of British Columbia, [3]CNRS, CSGA, [4]CNRS, IBDM

Animals use their sensory system to detect cues in their external environment and then communicate, process, and integrate, via the nervous system, these cues in order to induce a specific response. Taste is an important cue used by animals to explore their external environment and can modulate various aspects of behaviour and physiology in animals. A key ongoing challenge for animals is detecting and responding to the presence of a multitude of pathogens in their environment. However, to date, the links between the sensory system and the response to pathogenic threats remain poorly understood. Here we show that Drosophila larvae use their taste system to detect bacterial wall components in their environment and respond by modulating the activity of their cellular immune system. These results show that sensory inputs such as taste have an important role in protecting animals from bacterial infection. Overall, our findings add to the growing list of examples of crosstalk between the nervous system and the immune system and provide novel and important mechanisms for linking them.

21 **The TGFβ/Activin ligand Actβ is required to suppress glucose utilization by immune cells in the absence of infection** Heidi Bretscher, Michael B O›Connor University of Minnesota- Twin Cities

Metabolic homeostasis requires balancing nutrient utilization across multiple organ systems and preventing unnecessary activation of energetically expensive processes. One example of an energetically expensive process which must be tightly regulated is the immune response. Activation of the immune response is associated with large scale increases in glucose utilization by immune cells and a subsequent decrease in nutrient utilization and storage in peripheral tissues. In *Drosophila,* under basal conditions, the immune system consists of two cell types: plasmacytes, which are macrophage-like cells, and crystal cells, which are involved melanization and scaring. Both cell types are produced and differentiate in the lymph gland. In response to infection by a parasitic wasp, a subset of hematopoietic progenitors and circulating plasmacytes differentiate into lamellocytes, which are involved in encapsulation and melanization of the parasitic egg. We find that the TGFb/Activin ligand Activin b (Actb) is required to suppress metabolic activation of immune cells in the absence of a pathogen. Loss of Actb results in a net decrease in glucose catabolism, glycogen storage, mitochondrial activity and ATP generation in peripheral tissues and a very significant increase in glucose catabolism in immune cells. Specifically, we find a 400% increase in glucose incorporation into the Pentose Phosphate Pathway, a 50% increase in glycolysis and a 50% increase in polyol pathway/ sorbitol synthesis. In addition to metabolic changes in the immune cells, we find that Actb regulates immune cell differentiation. Loss of Actb results in an oversized lymph gland lacking crystal cells, and the surprising presence of lamellocytes in circulation. Together our work suggests that Actb regulates glucose and glycogen homeostasis via suppression of the immune response to parasitic wasp infection.

22 **Impairing mitochondrial function results in UPR^{mt} activation and improves survival outcomes after Flock House virus infection in *Drosophila melanogaster*** Dean Bunnell[1], Maddie Buhl[2], Grace Milas[2], Justin McGee[2], Stanislava Chtarbanova[2] [1]Biological Sciences, University of Alabama, [2]University of Alabama

A host's immune response and metabolic profile must be properly coordinated to ensure an effective response to infection. Our lab seeks to investigate how aging alters these processes that result in increased susceptibility to RNA virus infection relative to young hosts. Transcriptomics analysis showed that many genes whose products function in metabolic processes are regulated to a stronger extent in older flies following Flock House virus (FHV) infection. Using whole organism respirometry, we found that aged flies were not able to modulate their oxygen consumption rates (OCRs) after FHV infection, while young flies' OCR decreased longitudinally. We identified metabolism as a potential factor influencing older flies' accelerated mortality of FHV and sought to investigate if disruption of mitochondrial metabolism extends survival of FHV. Mitochondria's bacterial ancestry renders them susceptible to tetracycline and rifampicin antibiotics, which disrupt mitochondrial translation and transcription respectively causing mitochondrial dysfunction. Organisms survive infections by directly reducing pathogen load (resistance), and by limiting tissue damage caused by a pathogen or immune response (disease tolerance). In mammals, mitochondrial unfolded protein response (UPR^{mt}) activation has been shown to induce disease tolerance and improve survival outcomes of infection. Diverse mitochondrial stressors including reactive oxygen species or defects in oxidative phosphorylation can activate the UPR^{mt}, which in turn acts to resolve the appropriate stressor. We find that both tetracycline and rifampicin treatment extend survival of young and aged flies after FHV infection, independent of FHV loads when compared to each vehicle treatment. This suggests that survival extension likely results from improved disease tolerance. We found bacterial loads are not significantly different after FHV infection, confirming that protection occurs independently of antibiotic effects. Expression of *Hsp60,* a marker of UPR^{mt} activation, was significantly increased after tetracycline treatment in the aged, FHV-infected cohort. Finally, we find that mutants for *ND-23,* which encodes a component of the mitochondrial Complex I, outlive *wild type* controls following FHV challenge. Our results suggest that UPR^{mt} activation induces disease tolerance that extends survival of virus infection and identify host metabolism as a target for therapeutic interventions.

23 ***Drosophila* gut symbiont-host specificity is driven by selective adhesion** Kevin O Aumiller[1,2], Karina Gutierrez-Garcia[2], Ren Dodge[2], Ann Deng[1,2], Sneha Agrawal[1], Xincheng Yuan[1], William Ludington[1,2] [1]Johns Hopkins University, [2]Carnegie Institution of Washington

Animals form symbiotic relationships with resident gut microbiota that are both stable and host-specific. The current consensus in the field is that host specificity is achieved by environmental filtering, wherein the host gut provides a unique set of growth conditions for symbiotic bacteria that facilitate their colonization. We propose a higher degree of biological sophistication, where specificity is defined by co-evolved binding interactions mediated by bacterial adhesins. Lactobacilli are prevalent symbionts in mammals and insects such as the fruit fly *Drosophila melanogaster*. We isolated a strain of *Lactiplantibacillus plantarum* (*LpWF*) from wild *D. melanogaster* that colonizes a defined spatial niche within the foregut. We identified the genetic determinant of *LpWF* colonization through *in vitro* evolution and long-read sequencing: a genomic island that encodes two serine-rich repeat adhesins (SRRPs). Through CRISPRi knockdown experiments, we found that the SRRPs are each necessary to specify colonization of *L. plantarum* strains for distinct foregut subregions. Localization to the subregions was recapitulated by recombinant SRRP binding domains. Ectopic expression and cell surface display of the binding domains in mutant *L. plantarum* strains and the non-symbiotic bacterium *Lactococcus lactis* demonstrated sufficiency of these proteins to promote spatially-specific attachment to the foregut, suggesting that adhesion plays a key role in specifying colonization of the commensal niche. We currently aim to identify the Drosophila-expressed ligands of the SRRPs to further investigate the mechanisms by which animals establish and regulate specific host-microbe associations.

24 **Host-microbe cross-feeding determines host survival in a low nutrient context in *Drosophila*** Jason W Millington[1,2], Jamie A Lopez[2,3], Amin M Sajjadian[1], Robert J Scheffler[4], William B Ludington[4,5], Kerwyn Casey Huang[2,6,7], Lucy Erin O'Brien[1,7,8] [1]Molecular and Cellular Physiology, Stanford University, [2]Bioengineering, Stanford University, [3]Applied Physics, Stanford University, [4]Embryology, Carnegie Institution for Science, [5]Biology, Johns Hopkins University, [6]Microbiology and Immunology, Stanford University, [7]Chan Zuckerberg Biohub, [8]Stem Cell Biology and Regenerative Medicine, Stanford University

Every animal to exist has become permanently associated with intestinal microbes during, or shortly after, birth. This lifelong association of a host with its resident intestinal microbiota kickstarts a complex series of metabolic interactions with consequences for host metabolism, such as dietary energy acquisition, storage, and mobilization. Intestinal microbes compete for host-ingested nutrients; simultaneously, microbes engage in cross-feeding, generating metabolic byproducts used by the host. These microbe-derived metabolites impact host metabolism directly as nutrients but also as signaling molecules affecting global changes to metabolism. Here we show that host-microbe cross-feeding in *Drosophila* determines host survival in a low nutrient context. Using a minimalist, *Drosophila*-based assay we found that *Lactiplantibacillus plantarum*- (*Lp*) derived metabolites promote host survival in a low nutrient context beyond mathematical energy balance model predictions, suggesting a global effect on metabolism. We identify *Lp*-derived lactic acid as the cue promoting survival via host sensing of the relative concentrations of lactic acid to glucose. Together, these findings identify host-microbe cross-feeding via microbe-derived metabolites as key determinants of survival in response to environmental nutrient availability through global effects on metabolism.

25 **Fly tumors can evade cellular immunity by modulating basement membrane degradation** Kavya Adiga, Yoshiki Sakai, David Bilder Molecular and Cell Biology, University of California, Berkeley

Immune cells interact with tumors, and these interactions are important drivers of tumor growth and host lethality. Mammalian tumors acquire ways to evade the immune system; however, it is unknown if fly tumors can do the same. We show that a Drosophila ovarian carcinoma model recruits hemocytes and that the presence of tumor-associated hemocytes slows tumor progression and increases host lifespan. Interestingly, ovarian tumors secrete a GPI-linked protease inhibitor called Thioester Binding Protein 3 (Tep3), which restricts hemocyte recognition of the tumor and thus accelerates tumor progression and host death. Our data suggest that Tep3 inhibits the matrix metalloproteinase MMP1 to prevent tumor basement membrane breakdown, a known recruitment signal for hemocytes, and thus limit immune restriction of the tumor. I will discuss Tep3's role in immune evasion and how hemocytes affect tumor progression.

26 **Energetic Demands Regulate Sleep-Wake Rhythm Circuit Development** Amy R Poe[1], Lucy Zhu[2], Si Hao Tang[2], Ella Valencia[2], Matthew S Kayser[2] [1]Biological Sciences, University of Arkansas, [2]University of Pennsylvania

Normal sleep and circadian rhythms during early life are important for brain development. Indeed, disruptions in sleep and rhythms during development are a common co-morbidity in neurodevelopmental disorders including ADHD and autism. Although the molecular mechanisms encoding cellular rhythms are well understood, little is known about how rhythmic behaviors first emerge. We previously determined that sleep-wake rhythms are initiated in early 3rd instar *Drosophila* larvae (L3), coordinated through maturation of a circuit bridge connecting DN1a clock neurons and Dh44 arousal output neurons. Development of this circuit promotes deeper sleep, and related long-term memory (LTM) capabilities. The cues that trigger formation of this circadian sleep circuit are not known. Here, we demonstrate that changes in energetic demands during development drive maturation of behavioral patterns in sleep and feeding. During the 2nd instar (L2) period, sleep and feeding are spread across the day; these behaviors become organized into daily patterns by L3, with feeding consolidated to day and sleeping to night. Genetic and nutritional manipulations that force mature (L3) animals to adopt immature (L2) feeding strategies disrupt sleep-wake rhythms, leading to impaired sleep depth and deficient LTM capacity. We find that inducing deeper sleep stages in immature (L2) animals through pharmacological or genetic manipulations is energetically disadvantageous at this stage of life and does not improve LTM performance. Moreover, DN1a-Dh44 circuit formation itself is developmentally plastic: an insufficient nutritional environment prevents establishment of a functional connection, facilitating a more constant (strategic) feeding strategy that eschews deep sleep at the expense of LTM. Finally, Dh44 neurons act through glucose metabolic genes to sense an organism's nutritional environment and drive sleep-wake rhythm development. Together, our data demonstrate that the emergence of rhythmic behaviors in *Drosophila* is driven by developmental changes in energetic capacity.

27 **Taste cells expressing *Ionotropic Receptor* 94e impact multiple behaviors in *Drosophila melanogaster*** Jacqueline Guillemin[1], Jinfang Li[2], Viktoriya Li[2], Sasha A.T. McDowell[2], Kayla Audette[1], Grace Davis[1], Meghan Jelen[2], Samantha E Gibbons[1], Samy Slamani[1], Liam Kelliher[1], Michael D Gordon[2], Molly Stanley[1] [1]Department of Biology, University of Vermont, [2]Department of Zoology, University of British Columbia

Contact chemosensation, known as taste, is indispensable for ensuring an organism thrives in its environmental niche. Taste functions as an initiating signal for many complex behaviors, and in *Drosophila melanogaster*, labellar gustatory receptor neurons (GRNs) are involved in feeding, mating, and oviposition. Though the function of many of the GRNs are well-defined, the role of GRNs expressing *Ionotropic Receptor 94e* (IR94e), was previously unknown. Using optogenetics and chemogenetics in addition to the new whole-brain connectome, in vivo calcium imaging, and CRISPR mutants, we defined the role of this population of sensory neurons and the IR94e receptor. Direct activation of IR94e GRNs produced mildly aversive feeding behaviors, but in the connectome, we identified an excitatory IR94e circuit reaching the oviposition descending neurons (oviDNs). We experimentally validated that IR94e neuron activation increases oviposition by using chemogenetics plus in vivo calcium imaging and ovipositional assays, describing a novel role of labellar GRNs in egg laying behaviors. In vivo calcium imaging revealed that amino acids, specifically glutamate, produced calcium responses in IR94e neurons, and this response is dependent on *IR94e* expression. Finally, *IR94e* knock-in mutants exhibit reciprocal feeding and egg-laying behavior when encountering amino acid containing tastants, leading to the hypothesis that *IR94e* is necessary for the behavioral switch between feeding behavior and oviposition. In recent work we have started to investigate the role of IR94e in virgin females compared to mated females and have identified a change in activity that is unique to mated females. In conclusion, our studies identify the function of a unique set of labellar GRNs expressing *IR94e*, one that mediates a behavioral switch between feeding and egg-laying and may be impacted by internal state which leads to flexible behaviors to amino acid stimuli.

28 **Triggering and modulation of a complex behavior by a single peptidergic command neuron in *Drosophila*** Magdalena Fernandez-Acosta[1], Rebeca Zanini[1,2], Fabiana Heredia[1,2], Yanel Volonté[3], Juliane Menezes[1,2], Katja Prüger[1], Julieta Ibarra[3], Maite Arana[3], María Sol Perez[3], Jan A. Veenstra[4], Christian Wegener[5], Alisson M. Gontijo[1,2], Andres Garelli[1,2,3] [1]iNOVA4Health, Nova Medical School, NMS, Universidade Nova de Lisboa, [2]cE3c - Centre for Ecology, Evolution and Environmental Changes & CHANGE - Global Change and Sustainability Institute, Departamento de Biologia Animal, Faculdade de Ciências, Universidade de Lisboa, [3]Instituto de Investigaciones Bioquímicas de Bahía Blanca (INIBIBB), CONICET and Departamento de Biología, Bioquímica y Farmacia, Universidad Nacional del Sur., [4]INCIA UMR 5287 CNRS, Université de Bordeaux, [5]Julius-Maximilians-Universität Würzburg, Biocenter, Theodor-Boveri-Institute, Neurobiology and Genetics

At the end of their growth phase, Drosophila larvae remodel their bodies, glue themselves to a substrate, and harden their cuticle in preparation for metamorphosis. This process is termed pupariation and is triggered by a surge in the hormone ecdysone that initiates the behavioral sequence. Substrate attachment is achieved by a pupariation subprogram called glue expulsion and spreading behavior (GSB). An epidermis-to-CNS Dilp8-Lgr3 relaxin signaling event that occurs downstream of ecdysone is critical for unlocking progression of the pupariation motor program towards GSB, but the factors and circuits acting downstream of Lgr3 signaling remain unknown. Here, we screened for such factors using cell type-specific RNA interference and behavioral monitoring. We identify Myoinhibiting peptide (Mip) and its highly conserved neuronal receptor, Sex peptide receptor (SPR), as a critical neuropeptidergic signaling pathway required to trigger and modulate multiple action components of GSB. Dissection of the GSB behavioral sequence indicates that Mip acts on multiple SPR-positive neuronal populations, which collectively define and pattern the sequence and timing of GSB actions. In addition, we find that Mip is specifically required in a pair of descending command neurons, whose optogenetic activation at a specific competence window triggers GSB-like behavior. Our results advance our molecular and cellular understanding of pupariation control, provide insight into conserved aspects of Mip-SPR signaling in animals, reveal the complexity of glue expulsion and spreading behavior control, and contribute to the understanding of how multi-step innate behaviors are coordinated in time and with other developmental processes through command neurons and neuropeptidergic signaling. This work was supported by the FCT (EXPL/BIA-BID/1524/2021; EXPL/BIA-COM/1296/2021; 10.54499/2022.03859.PTDC), ANPCyT, CONICET, UNS-PGI, and DFG.

29 **Understanding inter-organ communication underlying feeding initiation in juvenile *Drosophila melanogaster*** Cindy Reinger[1], Laura Blackie[2], Pedro Gaspar[2], Alexandra Medeiros Vieira da Silva[1], Hugo Gillet[1], Michele Sickmann[1], Dafni Hadjieconomou[3], Anissa Kempf[1], Irene Miguel-Aliaga[2], Markus Affolter[1], Martin Müller[1] [1]Biozentrum, University of Basel, [2]Francis Crick Institute, [3]Paris Brain Institute

A conserved interplay of complex processes like feeding and excretion is required to fulfill nutritional needs and survival of many organisms. In their earliest hours of life, feeding needs to be initiated, and accumulated waste products of development, the so-called meconium, must be excreted. Whilst significant progress has been made in understanding these two processes independently, no studies have attempted an integrated exploration of whether and how feeding and excretion are mechanistically coupled. We have used a combination of *Drosophila melanogaster's* powerful toolbox of classical genetics and modern methods to explore the mechanism underlying feeding initiation and meconium excretion *in vivo*. We established new experiments to investigate feeding and excretion behavior in freshly hatched wild type flies. Akin to neonatal mammals, *Drosophila* excretes its meconium in multiple steps shortly after hatching. Only after its (partial) excretion, the cascade of feeding initiation is triggered. Through experiments in which we prevent meconium excretion, we demonstrate that excretion is required for feeding initiation, pointing to a gut-to-brain circuit coupling both processes. Further, we have identified a *cis*-regulatory element related to the selector gene *apterous*, which disrupts this gut-to-brain circuit. Flies without this *cis*-regulatory element, the "Life Span Enhancer (LSE)", display an ileus-like physical obstruction in their hindgut which prevents them from excreting their meconium. Consequently, they do not interact with food, barely eat and show an increase in proboscis extension sleep, a deep sleep stage with a functional role in waste clearance. More intriguingly, their small intestine (which does not express *apterous*) becomes bloated and shortened and eventually decays. The chronology of these phenotypes is highly reminiscent of the hallmarks described for mechanical ileus in humans. Our study provides new insights into the mechanistic links between meconium excretion, feeding behavior and their links to survival. It also provides a novel gene and a genetically tractable entry point into elucidating the mechanisms coupling both processes.

30 **Coracle Mutants Reveal a Role for Glia in Maintaining Synaptic Integrity** Daniel T Babcock, Danielle Moreira, Tulika Malik, Kevin Garzillo Biological Sciences, Lehigh University

Maintenance of synaptic integrity is crucial for communication within the nervous system. While a great deal is now known regarding the mechanisms underlying synaptic growth and development, much less is known regarding synaptic maintenance. We recently identified Coracle, a critical component of septate junctions, in our screen for genes associated with maintaining synaptic integrity with age. Coracle mutants show synaptic overgrowth at adult NMJs along with a progressive loss of functional integrity. We found that knockdown of coracle in glia recapitulated mutant phenotypes, and that coracle is specifically required in Subperineural glia (spg) to maintain synaptic integrity. Spg-specific knockdown of coracle causes seizure-like behavior and disruption of the Blood Brain Barrier (BBB), suggesting that increased neuronal excitability could underlie these synaptic phenotypes. We found that increasing glutamate buffering capacity in coracle mutants prevented barrier disruption and maintained synaptic integrity, suggesting that excitotoxicity is a likely contributor to this synaptic dysfunction. Finally, we determined that the immunosuppressant activity of Rapamycin exerted neuroprotective effects in coracle mutants even with BBB disruption, highlighting the role of the inflammatory response in disrupting synaptic integrity. Together, our results demonstrate a crucial role for glia in maintaining the structural and functional integrity of synapses with age.

31 **Trithorax regulates long-term memory in Drosophila through epigenetic maintenance of mushroom body metabolic state and translation capacity** Jamie M Kramer Dalhousie University

The role of epigenetics and chromatin in the maintenance of postmitotic neuronal cell identities is not well understood. Here, we show that the histone methyltransferase Trithorax (Trx) is required in postmitotic memory neurons of the *Drosophila* mushroom body (MB) to enable their capacity for long-term memory (LTM), but not short-term memory (STM). Using MB-specific RNA-, ChIP-, and ATAC-sequencing, we find that Trx maintains homeostatic expression of several non-canonical MB-enriched transcripts, including the orphan nuclear receptor *Hr51*, and the metabolic enzyme *lactate dehydrogenase* (*Ldh)*. Through these key targets, Trx facilitates a metabolic state characterized by high lactate levels in MBγ neurons. This metabolic state supports a high capacity for protein translation, a process that is essential for LTM, but not STM. These data suggest that Trx, a classic regulator of cell type specification during development, has additional functions in maintaining underappreciated aspects of postmitotic neuron identity, such as metabolic state. Our work supports a body of evidence suggesting that a high capacity for energy metabolism is an essential cell identity characteristic for neurons that mediate LTM.

32 **A Sex-Specific Neuroligin-based Developmental Switch Regulates Presynaptic Site Formation** Kristen C Davis[1,2], Timothy Mosca[1,2] [1]Neuroscience, Thomas Jefferson University, [2]Vickie and Jack Farber Institute for Neuroscience, Thomas Jefferson University

Proper synaptic development is vital to optimal neural function but when impaired, neurodevelopmental disorders arise. Our understanding of how synaptic biology intersects such disorders like autism spectrum disorders (ASD) is inadequate as is our grasp of the genetic underpinning of male/female ASD diagnosis disparities. Multiple genes are linked to ASD, including two transsynaptic cell-surface molecules, *neurexin* (*nrx)* and *neuroligin* (*nlg*), that promote typical circuit development but whose role is yet unexplained. To better understand Nrx/Nlg in synapse development and ASD, we use the *Drosophila* antennal lobe (AL), a CNS circuit well suited for studying synapse development. Flies have one Nrx and four Nlg homologs; we examined each in regulating synapses between olfactory receptor (ORN) and projection (PN) neurons in the AL. We found all five genes are expressed in the AL; we observed sex-specific differences in *dnlg3* and *dnlg4* expression, a previously unappreciated variation. To understand how these genes act in development, we impaired Nrx and Nlg in presynaptic ORNs or postsynaptic PNs. We found presynaptic Nrx signals to postsynaptic Nlg (as expected) to promote synapse addition, but males and females use a different Nlg code to promote synaptogenesis, a novel sexual dimorphism. We also found that Nlg, thought to act only postsynaptically, also acts presynaptically to regulate synapse number, with a different presynaptic Nlg code in males and females. Interestingly, presynaptic Nlg responds to glial Nrx indicating a novel glial Nrx::presynaptic Nlg signaling axis in synaptogenesis. To find when presynaptic Nlgs act in synaptogenesis, we compared early knockdown to adult knockdown and found that loss of *dnlg1* and *dnlg3* in females or *dnlg3* and *dnlg4* in males unexpectedly increased synapse number. This suggests presynaptic Nlgs have a developmental switch, functioning early in promoting and later in restricting synapses. This resembles human data (autistic brains have more connections than neurotypical brains) and may also explain sex differences. Overall, we found key roles for Nrx and Nlg in synaptogenesis, a novel glial Nrx::presynaptic Nlg axis in synapse growth, and a developmental switch in presynaptic Nlg function, with sex differences in the Nlg code used by each cell. This provides notable insight into the complexity of synaptogenesis and sex, highlighting *Drosophila* as a system to test specific ASD hypotheses at synaptic resolution.

33 **Neural origin of a female behavioral novelty in *Drosophila*** Minhao Li[1], Dawn Chen[1], Ian P Junker[1], Fabianna I Szorenyi[2], Guan Hao Chen[2], Arnold J Berger[2], Aaron A Comeault[3,4], Daniel R Matute[3], Yun Ding[1] [1]Biology, University of Pennsylvania, [2]University of Pennsylvania, [3]Biology, University of North Carolina, Chapel Hill, [4]Environmental and Natural Sciences, Bangor University

Social interactions during courtship are highly diverse among animal species. How neural circuits evolve to generate behavioral novelty is yet to be depicted. We identified a newly originated female sexual behavior in the island endemic species, *Drosophila santomea*, where females spread their wings as a receptive signal to the males' courtship song. This wing spreading behavior is layered on top of the conserved female receptive behavior vaginal plate opening, and induces male to sing a longer song, forming a species-specific social feedback loop. Copulation success depends on this female signal and correlates with males' ability to adjust his singing. By comparing the function of homologous circuits across species by optogenetic activation, we identified that vpoDN, the female-specific descending command neurons that control vaginal plate opening, is recruited to also drive wing spreading in *D. santomea* females. Surprisingly, wing spreading can be induced idiosyncratically by vpoDN activation in females of the outgroup species *D. melanogaster*, suggesting that the ancestral circuit possesses the wing spreading potential. Furthermore, this potential is highly plastic and can be boosted by a higher developmental temperature. Consistent with the presence of a latent potential, we found that wing spreading-like behavior is occasionally expressed as extremely rare events during courtship interaction in wildtype *D. melanogaster* females. Finally, behavioral characterizations and ancestral reconstruction across a broad phylogeny suggested that wing spreading in *D. santomea* is a behavioral novelty without an ancestral homolog, and that wing spreading is a recurrently evolving signal in *Drosophila*. Overall, our results revealed that wing spreading evolved by actualizing the ancestral potential of a socially-tuned key circuit node to engage the wing motor program, facilitating the expression of this female novel behavior in appropriate sensory and motivational contexts. Leveraging EM connectome and functional imaging, we are characterizing the downstream circuit of vpoDN that accounts for the wing spreading latent potential in *D. melanogaster*, and how it has evolved to actualize this behavior in *D. santomea*. Together, our work provides insights into the neural underpinnings underlying the origin of behavioral novelty.

34 **The dynamics of Myosin reorganization in pupal wing expansion** Anni Yi[1], Kenneth D. Irvine[2] [1]Molecular Biology and Biochemistry, Rutgers University New Brunswick, [2]Molecular Biology and Biochemistry, Waksman Institute of Microbiology

During the pupal stage of Drosophila melanogaster, it is known that the pupal wing flattens and expands to form the adult wing and that pupal wing cells change shape and flatten during this expansion. However, this crucial step in pupal development is still not very well understood in its early stages. Non-muscle Myosin II, encoded by the gene Spaghetti Squash in Drosophila, has been shown to have structural functions within the cell helping to facilitate cell migration and cell division. Here, we are investigating its behavior during pupal wing expansion. We examined Myosin II's localization in fixed pupal wings of various ages using fluorescent protein-tagged Myosin II transgenes, as well as investigated its dynamics and relocalization by performing in vivo time lapse imaging. While previous studies have shown that Myosin II relocalizing to the lateral sides of the cell from 6-8 hours post pupariation plays a role in pupal wing expansion, our results suggest that there may be additional mechanisms that control the cell shape changes involved in pupal wing expansion. Our results have shown transient rapid "movement" of partially lateral Myosin unevenly distributed throughout the pupal wing. We also found that multiple adherens junctions proteins and Beta spectrin colocalize with Myosin where it is enriched laterally. In addition to these two findings, our results showed that transient local invaginations in the apical surface of the wing correlates with areas of lateral Myosin enrichments and shorter cells. Further research into the potential relationships between the dynamic Myosin relocalization, local invaginations, and adherens junctions proteins and Beta spectrin colocalization with Myosin could provide new insight into the biomechanical controls of the early stages of pupal wing development.

35 **Targeted RNA interference Screen to Identify Novel Modifiers of Huntington's Disease impact on adult viability in *Drosophila*** Sevinch U Kamaridinova, Tadros A Hana, Kiel G Ormerod Biology, Middle Tennessee State University

Huntington's Disease (HD) is an inherited neurodegenerative disorder highlighted by progressive breakdown in neurons and leading to progressive loss of motor control. Unlike other neurodegenerative disorders, HD research is focused mostly on one gene, the Huntingtin (HTT) gene. The disease is attributable to abnormal expansion of the CAG trinucleotide repeat region found in HTT, causing the htt protein product to contain an aberrant number of glutamines (ref). This increased length of the polyglutamine tract (PolyQ) leads to instability that leads to a misfolded, aggregation phenotype associated with HD (ref). The severity of the disease and the age of onset have been shown to correlate with the degree of expansion within the PolyQ region of htt where an increase in glutamine repeats increases the pathogenicity and reduces age of onset (ref). A transgenic *Drosophila* model of HD was previously created in the Littleton laboratory, where a truncated version of human htt was genetically altered to include either 15 or 138 glutamine repeats within the PolyQ region (non-pathogenic htt-Q15, pathogenic htt-Q138). These transgenic lines also included an N-terminal RFP-tag for fluorescent imaging. Flies expressing htt-Q138 in motor neurons (Elav-Gal4) were previously shown to have a dramatically reduced lifespan compared to those expressing htt-Q15. Here we expressed these htt-constructs using Elav-Gal4 along with RNA interference to selectively target 29 different genes associated with HD pathology or htt functionality and looked for enhancers or suppressors of the lifespan phenotype. The results of the genetic screen herein could identify potential therapeutic targets for novel HD treatment or can shed light on the underlying disease mechanisms of HD.

36 ***Drosophila melanogaster* Genotype Impacts Metabolic Response to Chronic Bacterial Infection** Ananda A Kalukin[1,2], Scott A Keith[1,2], Andrea M Darby[1,2], Brian P Lazzaro[1,2] [1]Entomology, Cornell University, [2]Cornell Institute of Host-Microbe Interactions and Disease

Infections have a significant impact on host metabolism as hosts must mobilize energy to repair damage and produce an immune response. By characterizing chronic infections in *Drosophila* across a panel of both Gram-negative and Gram-positive bacterial strains, we found that the magnitude of chronic bacterial load has a profound effect on *Drosophila* metabolism. Higher chronic pathogen burdens resulted in higher starvation susceptibility, regardless of whether the higher burden was established through higher initial inoculation dose of a given pathogen or through infection with bacterial strains that naturally establish higher chronic burdens. This effect may be partially due to the energetic cost of upregulating immune genes like antimicrobial peptides (AMPs), as we found that activation of the immune response even in the absence of infection was sufficient to reduce starvation resistance. We next hypothesized that naturally diverse host genotypes would sustain different chronic bacterial loads, which in turn might result in differential metabolic costs which would be revealed in starvation sensitivity. To evaluate this, we sampled five strains from the *Drosophila* Genetic Reference Panel and used a ring cross to establish heterozygous progeny. We infected these outbred genotypes with *Providencia rettgeri* and *Serratia marcescens*. Contrary to our first prediction, chronic loads of each pathogen were comparable across fly genotypes. However, the genotypes varied in the rate at which their metabolic stores were depleted during the chronic phase of infection, suggesting variable infection tolerance. Additionally, we found that genotypes with lower expression of genes encoding antimicrobial peptides (AMPs) prior to infection were more likely to die from infection, suggesting variation in constitutive resistance. These genotypes also showed lower triglyceride levels in the chronic infection phase of survivors. Thus, our data show evidence for variation in resistance as determined by constitutive immune system activity, as well as variation in tolerance measured as metabolic cost during the chronic phase of infection.

37 **Coactivator crosstalk: Yorkie and Taiman interact to regulate germline niche-to-stem cell signaling** Chloe Wells, Victoria Placentra, Shilpi Verghese, Ken Moberg Cell Biology, Emory University

Communication between stem cells and the specialized niches they reside in is vital for organism development and tissue homeostasis. However, pathways that act within a given niche to guide stem cell fates and regulate differentiation of their progeny are not fully understood. The Hippo and Ecdysone nuclear receptor (EcR) signaling pathways have each been shown to regulate stem cell niches in multiple *Drosophila* tissues, and both are also implicated in human disease. Significantly, these two pathways are linked in the nucleus via a direct, physical interaction between their respective transcriptional coactivators Yorkie (Yki) and Taiman (Tai), and this interaction appears conserved in mammals. Within the female *Drosophila* germline niche (e.g. the germarium), Yki and Tai have separately been shown to regulate behavior of stem cells and their progeny, but it is not known whether these roles stem from crosstalk between the Hippo and EcR pathways, or whether these roles are mechanistically distinct. Here we have utilized a CRISPR mutant fly line containing a form of Tai unable to bind Yki (Tai^{PPxA}) to examine requirements for the Yik-Tai interaction *in vivo*. Our data indicate that loss of this interaction in Tai^{PPxA} flies decreases female fertility and reduces the number of developing egg chambers. At a cellular level, mutant germaria contain increased numbers of cells with single “dot” spectrosomes, suggesting that loss of Yki-Tai binding impairs germline stem cell differentiation and prevents appearance of differentiating cytsoblasts with branched fusomes. To map which germarium cell types require the Yki-Tai interaction, we have used the Gal4-UAS system and found evidence that expression of Tai^{PPxA} in supporting escort cells is sufficient to replicate the germline impairment of fully Tai^{PPxA} mutant ovaries. Experiments are ongoing to identify Yki-Tai transcriptional targets produced in escort cells that act on germline cells. In sum, these findings suggest that a Yki-Tai interaction may facilitate crosstalk between the Hippo and EcR pathways within escort cells in the female germline, and that this interaction is critical to maintain intracellular signals that allow escort cells to guide early stages of egg development.

38 **Regulation of autophagic cell death by intracellular pH and the proto-oncogene *Myc*** Alan Wong[1], Tiana Tameta-Arenas[1], Antonio Bibiano[1], Kimberly Nguyen[1], Jobelle Peralta[2], Blake DuPriest[1], Daniel Orozco[1], Juan Reyna Pacheco[1], Laura Martins[1], Rachel Ann Soriano[1], Ramy Wong[1], Bree Grillo-Hill[1] [1]San Jose State University, [2]University of Washington

Increased intracellular pH (pHi) is common to most cancers regardless of the 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. One objective of our current work is to understand how pHi dynamics influence growth control during development, as previous studies suggest that higher pHi promotes proliferation and inhibits apoptotic cell death. Our lab developed tools to increase pHi in the absence of transforming mutations by over-expressing the Drosophila sodium-proton exchanger, *DNhe2*, in the developing eye. We previously showed that flies overexpressing *DNhe2* have a "rough" or mispatterned adult eye. To determine the underlying cause of this rough eye, we examined fly eyes at two earlier stages of development (larval and pupal), and counted proliferating cells and quantified total cell numbers. We previously showed that flies overexpressing *DNhe2* show increased proliferation in larval eye discs. Paradoxically, we found fewer cells at the end of patterning in pupal eyes (11.4 per counting area compared to 15 cells in control). We next tested whether this decrease in cell number was due to increased cell death. We found that the pH-dependent cell death is caspase independent but required the autophagy genes *Atg1*, *Atg7* and *Atg8a*, which is inconsistent with apoptosis but suggests autophagic cell death. We also found that molecular markers for autophagy support increased autophagy at higher pHi. Next, we want to identify the genes that mediate increased autophagy at higher pHi. A dominant modifier screen identified overexpression of the proto-oncogene *Myc* as a strong suppressor of the *DNhe2*-induced rough eye. We tested whether this suppression is due to Myc attenuating the hyperproliferative effects of over-expressing *DNhe2*, but we saw no effect. However, we found that co-expression of *Myc* inhibits the autophagic cell death seen with over-expression of *DNhe2*. We are currently working to determine the molecular mechanism by which pHi regulates *Myc*, and how *Myc* decreases autophagy. Together, our findings elucidate mechanisms for pH-regulation of conserved, critical developmental processes and provide evidence for new paradigms in growth control.

39 **Mechanisms of Glyphosate-Based Herbicide Toxicity on *Drosophila* Gonads** Randy Bracamontes[1], Samantha Srisamai[2], Priscilla Estrada[2], Mike Rizzo[3], Anna Zelaya[2], Becky Talyn[4], Erik Johnson[3] [1]Biology, Riverside City College, [2]Biology, California State University, [3]Biology, Wake Forest University, [4]College of Natural Sciences, California State University

The widespread use of glyphosate and glyphosate-based herbicides (GBHs) like Round-Up has sparked significant concern over their potential effects on non-target organisms. Glyphosate works by inhibiting the enzyme 5-enolpyruvylshikimate-3-phosphate (EPSP) synthase, thereby disrupting the Shikimic acid pathway, which is essential for the synthesis of amino acids vital for protein production and plant growth. While this mechanism targets plants, leading to their eventual death, this study investigates the broader implications of GBH application by focusing on *Drosophila melanogaster*, a non-target species. Consistent with previous studies, we hypothesized that exposure to GBH would reduce the size of ovaries, and extend this work to include testes. In addition, we examine the role of two potential mechanisms, the juvenile hormone pathway and the reproductive microbiome.

1. To examine effects on ovary and testes size, adult *D. melanogaster* were exposed to varying concentrations of Round-Up and glyphosate alone over a period of 7 days. Gonads were removed from the body and measured using a DinoLite camera on a trinocular dissecting microscope and DinoLite software. Exposure to GBH reduced the size of both ovaries and testes, but glyphosate exposure did not affect testes size as dramatically.

2. The role of JH was examined by treating newly eclosed adults with methoprene dissolved in acetone or an acetone control before exposure to GBH or glyphosate. While both GBH and glyphosate decreased ovary size, methoprene did not affect this. The results for testes were more complicated.

3. We began to explore the role of the reproductive microbiome by dissecting ovaries and testes under sterile conditions into nutrient broth. Microbial colonies were successfully isolated from both ovaries and testes, indicating the presence of a reproductive microbiome in both female and male *Drosophila*.

Along with other experiments conducted in our lab, this work shows significant reductions in gonad size and reproductive success, altered egg-laying behavior, and abnormal locomotor activity, suggesting that glyphosate exposure induces physiological stress and endocrine disruption in *D. melanogaster*. These findings contribute to the growing body of evidence that the toxicity of GBHs extend beyond plants utilizing the Shikimic pathway, impacting a broader range of species and raising concerns about the ecological consequences of glyphosate-based herbicides and highlighting the need for further studies on its long-term environmental effects.

40 **Affinity hierarchies and amphiphilic proteins underlie the co-assembly of nucleolar and heterochromatin condensates** Varsha Rajshekar[1], Omar Adame-Arana[2], Gaurav Bajpai[3], Samuel Safran[4], Gary Karpen[5] [1]Molecular and Cell Biology, UC Berkeley, [2]Max Planck Institute for the Physics of Complex Systems, [3]Northeastern University, [4]Weizmann Institute of Science, [5]UC Berkeley

Nucleoli are surrounded by Pericentromeric Heterochromatin (PCH), reflecting a close spatial association between the two largest biomolecular condensates in eukaryotic nuclei. Nucleoli are the sites of ribosome synthesis, while the repeat-rich PCH is essential for chromosome segregation, genome stability, and transcriptional silencing. How and why these two distinct condensates co-assemble is unclear. Here, using high-resolution live imaging of *Drosophila* embryogenesis, we find that *de novo* establishment of PCH around the nucleolus is highly dynamic, transitioning from the nuclear edge to surrounding the nucleolus. Eliminating the nucleolus by removing the ribosomal RNA genes (rDNA) resulted in increased PCH compaction and subsequent reorganization into a toroidal structure. In addition, in embryos lacking rDNA, some nucleolar proteins were redistributed into new bodies or 'neocondensates', including enrichment in the PCH toroidal hole. Combining these observations with physical modeling revealed that nucleolar-PCH associations can be mediated by a hierarchy of interaction strengths between PCH, nucleoli, and 'amphiphilic' protein(s) that have affinities for both nucleolar and PCH components. We validated this model by identifying a candidate amphiphile, a DEAD-Box RNA Helicase called Pitchoune, whose depletion or mutation of its PCH interaction motif disrupted PCH-nucleolar associations. Together, this study unveils a dynamic program for establishing nucleolar-PCH associations during animal development, demonstrates that nucleoli are required for normal PCH organization, and identifies Pitchoune as an amphiphilic molecular link required for PCH-nucleolar associations.

41 **Prostaglandin signaling promotes nucleoskeletal changes required for on-time border cell migration** Ashley C Goll[1], Kaden Bex[2], Ellie Nacino[2], Tina L Tootle[1] [1]Biology, University of Iowa, [2]University of Iowa

Cell migration is required for many biological processes including development, wound healing, and cancer. To migrate through 3D space, the cytoskeleton rearranges and transmits mechanical signaling to the nucleoskeleton, driving changes in nucleoskeletal composition to ultimately control nuclear stiffness. Key components of the nucleoskeleton are Lamin A, Lamin B, and Emerin. Reducing Lamin A results in a softer, whereas increasing Lamin A and Emerin causes a stiffer nucleus. While nuclear stiffness regulates 3D single cell migration, little is known its roles in 3D collective cell migration. To address this unknown, we use Drosophila border cell migration as an *in vivo* model of 3D collective cell migration. During Stage 9 of oogenesis polar cells designate 6 to 8 outer follicle cells to become border cells, which migrate as a group or cluster from the anterior end of a follicle between the nurse cells to the oocyte. During Stage 8, Lamin B, Lamin A, and Emerin are prevalent in all the outer follicle cells. Upon border cell specification, Lamin B remains high throughout the cluster, whereas Lamin A is diminished in the border cells but high in the polar cells; this pattern remains throughout migration. Conversely, early in migration Emerin is prevalent in the polar cells and border cells but becomes restricted to the polar cells by mid-migration. These data suggest that during specification the border cell nuclei become softer to facilitate migration. Indeed, overexpression of Lamin A in the border cells results in increased levels of Emerin and delays migration. We next sought to identify the mechanisms controlling nucleoskeletal remodeling during border cell migration. As previous work found that prostaglandins (PGs), short range lipid signaling molecules, promote on-time border cell migration, we asked if PGs regulate the nucleoskeletal changes during border cell migration. Loss of all PG synthesis has no striking impact on Lamin B, but results in high Lamin A and Emerin in the border cells throughout migration; these finding suggests that the nuclei are stiffer and this increase in stiffness may contribute to the delay in migration. Together these results suggests that PG signaling regulates the nucleoskeleton to soften the border cell nuclei to facilitating their 3D collective cell migration. Similar PG regulation of nuclear stiffness likely facilitates collective cell migration across organisms, including during wound healing and cancer metastasis.

42 **Formation of energid, the non-membrane bound psuedo-cell compartments in the syncytial Drosophila embryo** Chase J. Yezzi, Mo Weng, Lingkun Gu, Ferdos Fessahaye School of Life Sciences, University of Nevada, Las Vegas

Cellular compartmentalization processes, such as spatial placement of organelles, are essential for cell survival and function. However, the molecular mechanism of spatially segregating cell components, especially the non-membrane-bound compartments, is still elusive. Drosophila embryo begins development with nuclear division without cytokinesis, making one large syncytial cell. It was previously proposed that these syncytial nuclei are surrounded by cytoplasm to form pseudo-cell compartments called energids, but the content of the energid has yet to be molecularly defined, and the mechanism of their assembly and maintenance is unknown. Using cytosol and organelle markers, we found that the syncytial embryo interior is initially a homogenous mixture of yolk and cytoplasm. The cytoplasm portion contains cytosolic proteins and organelles, primarily the tubular-like endoplasmic reticulum, distributed throughout the entire embryo. We found that the energid forms as soon as the first nuclear cycle and contains the same cytoplasmic components as the surrounding mixture. Using embryo explants, we observed that energids form through the segregation of cytoplasm from the homogenous mixture surrounding the dividing nuclei with ER tubules absorbed into the newly forming energid. As nuclei continue to divide and form energids, the space they occupy expands to the entire embryo, and the interior of the embryo becomes compartmentalized into individual energids separated by yolk materials. Importantly, we identified centrosomes as the main organizer of the energid cytoplasm: centrosomes dissociated from the nuclei or chromatin are sufficient to segregate and organize cytosol and ER into energid-like compartments. While centrosomes are needed to establish the energid organization, we found that complete separation of dividing energids is essential for their maintenance. Loss of Gish, a casein kinase 1γ homolog, leads to incomplete energid divisions, resulting in irregular spacing and fusion between energid compartments, and ultimately the breakdown of their corresponding nuclei. Overall, our results show that the initial homogeneous mixture of yolk and cytoplasm of embryo interior is compartmentalized into energid units by segregating cytoplasmic components from the yolk through the centrosome organizers; once assembled, they need to be kept apart, which requires the conserved kinase, Gish.

43 **Activating adaptors are not essential for Dynein-mediated microtubule gliding during *Drosophila* oogenesis** Phylicia Allen[1], Wen Lu[2], Rajalakshmi Veeranan-Karmegam[1], Hannah Neiswender[1], Daniela Domkam[3], Graydon Gonsalvez[1], Vladimir I Gelfand[2] [1]Cellular Biology and Anatomy, Medical College of Georgia, [2]Cell and Developmental Biology, Northwestern University, [3]Medical College of Georgia

Cytoplasmic Dynein-1 (Dynein) is the motor responsible for most retrograde cargo transport in eukaryotes. Dynein is a large dimeric complex made up of multiple proteins: Dynein heavy chain (Dhc), Dynein intermediate chain (Dic), Dynein light intermediate chain (Dlic), and three types of Dynein light chains (Dlc). Dynein activity requires the large Dynactin complex. In vitro studies indicate that activating cargo adaptors, which link Dynein with cargo, are also essential for fully activating the motor. The current model suggests that cargo adaptors stabilize the trimeric Dynein-Dynactin-adaptor complex. The goal of the present study was to use the egg chamber of the *Drosophila* model system to examine the role of activating cargo adaptors in stimulating Dynein-mediated transport. During egg chamber maturation, cargo transport from the nurse cells into the oocyte is mediated by Dynein. Thus, perturbing Dynein activity produces phenotypes that are easy to characterize and quantify. Previous structural and mutagenesis studies using mammalian Dlic identified residues that were critical for interaction with cargo adaptors. We therefore constructed transgenes in which these conserved amino acids were mutated. Depletion of Dlic in the germline results in an oogenesis block. Expression of wild-type Dlic completely restored oogenesis. Surprisingly, expression of the Dlic mutants also restored oogenesis. As expected, however, the Dlic mutants were significantly compromised in binding BicD, a highly conserved cargo adaptor. Consistent with this defect, and with a disruption of Dynein-mediated cargo transport, the oocyte enrichment of Dlic, Dhc, and BicD were reduced. The oocyte enrichment of BicD-dependent protein and mRNA cargos were also reduced. Thus, the Dlic-adaptor interaction is critical for efficient cargo transport into the oocyte. Another Dynein dependent function is microtubule gliding. Gliding creates advection forces that non-specifically delivers cargo into the oocyte, promoting oocyte growth. Surprisingly, this aspect of Dynein function was not affected in the Dlic mutant background. In addition, contrary to the current model, loss of cargo adaptors did not affect the stability of the Dynein-Dynactin interaction. Our results therefore suggest that cargo adaptors are most critical for specific cargo transport but are not essential for microtubule gliding or stabilizing the Dynein-Dynactin interaction.

44 **Dissecting the mechanistic links between cell chirality and chiral actin fibers formed by class I myosins** Daiki Kitamura[1], Haruna Nishikawa[1], Mikiko Inaki[1,2], Kenji Matsuno[1] [1]Department of Biological Sciences, Graduate School of Science, The University of Osaka, [2]Department of Life Science, Graduate School of Science, University of Hyogo

Many organisms show left-right (LR) asymmetry in their body structures and functions. This formation is a genetically regulated process, and its disruption can result in syndromes such as heterotaxy in humans. Although the mechanisms for LR asymmetry formation are well-studied in vertebrates, they are still elusive in invertebrates. It has been shown that "cell chirality", which is an LR-asymmetric property in the shape and behavior of cells, drives organ LR-asymmetry formation in invertebrates including *Drosophila*. However, the mechanism for cell chirality generation is unknown. Recently, overexpression of class I myosins, Myosin ID (MyoID) and Myosin IC (MyoIC), in the larval epidermis has been reported to induce left-handed and right-handed larval body twisting, respectively. The former and latter are coupled with clockwise and counterclockwise rotations of the epidermal cells, respectively. In the larval epidermal cells of these larvae, we detected F-actin using Lifeact-GFP and found that MyoID and MyoIC overexpression converted F-actin into long fibers tilted in the upper left and right, respectively. We hypothesized that these chiral F-actin fibers generate mechanical forces to induce chiral deformation of the cells. To test this hypothesis, we first conducted a genetic screen to explore genes involved in the chiral F-actin fiber formation. In this screen, we overexpressed both MyoID and Lifeact-GFP in the epidermal cells and simultaneously knocked down actin-related genes, and successfully identified genes whose knockdown inhibited the chiral F-actin fiber formation. We are now analyzing changes of the cellular shape and larval body twisting under these conditions. Additionally, we are also performing a laser ablation experiment to measure the tension applied on the chiral F-actin fibers, which will be discussed with the screening data.

45 **A divergent, testis-enriched actin paralog plays roles beyond the germline** Courtney M Schroeder[1], Kaitlin Stromberg[2] [1]Pharmacology, UT Southwestern Medical Center, [2]UT Southwestern Medical Center

The actin and actin-related protein (Arp) superfamily is ancient and under stringent sequence conservation for many critical cellular processes in eukaryotes. However, we also see recent diversification of the Arp superfamily across phyla. *Arp53D* is a recently evolved Arp found in most *Drosophila* species and is a model for studying Arp diversification. Unlike the ubiquitously expressed canonical Arps, *Arp53D* is highly enriched in the testis, where it localizes to specialized actin structures. Previously, we found that *Arp53D* knockouts (KOs) have increased male fertility while *Arp53D*-KO females are sub-fertile under heat stress, indicating it functions beyond the male germline. This unexpected female phenotype fits with the "out-of-testis" hypothesis, which predicts that the testis provides an initial "playground" for evolutionary innovation and advantageous diversification then broadens its tissue expression. Here, we investigated the role of *Arp53D* in females and found that it impacts oogenesis. Eggs develop in a series of egg chambers, and in *Arp53D*-KO females, a significant number of egg chambers undergo apoptosis during a critical nutrient checkpoint. Because we could not detect Arp53D protein or mRNA in ovaries, we explored whether Arp53D distally impacts oogenesis and is expressed beyond the female germline. RNA-seq and proteomics data indicate that Arp53D is expressed in the fat body, a metabolic hub equivalent to human adipose tissue and the liver. The fat body is a critical organ in female fertility as a source of proteins and fats for oogenesis and subsequent progeny development. We found that lipid droplets in *Arp53D*-KO fat bodies are significantly larger than those in wildtype flies, suggesting a defect in lipolysis. Furthermore, fat body-specific expression of *Arp53D* in KOs rescues egg chamber death. Together, these data suggest that Arp53D is important for intra-organ nutrient trafficking. Interestingly, when we encoded Arp53D's unique, rapidly evolving N-terminus on actin, we found that this chimera partially rescues the phenotypes we observe in females, indicating that Arp53D behaves similarly to actin, yet its N-terminus makes it functionally distinct. To further study Arp53D mechanistically, we purified it, and our preliminary data shows it directly binds filamentous actin *in vitro*. Our findings suggest that Arp53D plays unexpected roles in actin biology beyond the germline.

46 **Mapping promoter-enhancer interactions at the *Drosophila shavenbaby* locus** Sujay Naik, Ella Preger-Ben Noon Department of Genetics and Developmental Biology, Technion

Gene regulation is fundamental to every aspect of life. The onset of tissue-specific gene expression depends on the function of genomic elements called enhancers. Enhancers activate gene expression by interacting with gene promoters, often located at great genomic distances through long-range chromosomal interactions. A longstanding question in enhancer biology is how these physical interactions between enhancers and promoters are established and regulated across different developmental contexts.

Here, we use the *shavenbaby* gene as a model system to elucidate mechanisms of enhancer-promoter interactions during *Drosophila* development. *Shavenbaby* encodes a transcription factor that directs cuticular trichome development. Its embryonic expression is regulated by the combined activities of seven enhancers scattered in a 90-kilobase gene desert upstream of the *shavenbaby* promoter. We performed cell type-specific chromosome conformation capture sequencing (4C-seq) to map genomic interactions of the *shavenbaby* promoter and enhancers across different cellular and developmental contexts.

In the embryo, we find that the *shavenbaby* promoter interacts with all known *shavenbaby* enhancers in all tested epidermal subpopulation. Interestingly, beyond interactions with the promoter, all *shavenbaby* enhancers also interact with each other, even in epidermal cells where specific enhancers are inactive. In the pupal epidermis, we observe that the *shavenbaby* promoter forms a higher density of interactions with its regulatory region compared to the more discrete interactions observed in the embryonic epidermis. These findings suggest that the *shavenbaby* enhancers and promoter form a hub that persists across diverse trans-regulatory landscapes and may contribute to regulatory robustness and adaptability across developmental stages in *Drosophila*.

47 **3D dynamics of *trans* enhancer-promoter interactions in living *Drosophila* embryos reveals spatiotemporal thresholds for transcription activation** Bomyi Lim[1], Hao Deng[2], Philippe Valenti[3], François Payre[3] [1]Chemical and Biomolecular Engineering, University of Pennsylvania, [2]University of Pennsylvania, [3]Université de Toulouse

While it is well acknowledged that specific enhancer-promoter interactions are essential for transcription, the spatiotemporal thresholds required for transcription initiation remain unclear. Should enhancer and promoter be in close proximity for a sustained amount of time, or is a transient “kissing” sufficient? Here, we employed the phenomenon of transvection, where an enhancer activates the target gene in *trans* at the homologous position, to analyze the mechanism of long-range enhancer-promoter interactions. We combined the MS2- and PP7-based RNA labeling with the ParB/*parS* DNA labeling to simultaneously visualize active transcription and a target DNA locus in living *Drosophila* embryos. By quantifying the 3D enhancer-promoter distances in nuclei that exhibited active or inactive transcription, we found that sustained proximity between the enhancer and promoter is necessary and sufficient to initiate transcription. While transient enhancer-promoter interactions occured frequently, it was not sufficient to drive active transcription. The enhancer-promoter proximity was established before transcription initiation and remained stable after the termination, suggesting the localization of an enhancer and the target promoters in a “transcription hub.” When transvection occurred, the *cis*-linked reporter gene showed a delayed activation and reduced mRNA production, suggesting homologous promoter competition in the hub. Our DNA-labeled co-transvection assay sheds insights into the activation criteria for the long-range enhancer-promoter interactions and provides a stepping stone for future endeavors to understand the complex gene regulation in 3D space.

48 **Essential, pioneering features of the conserved transcription factor Grainy head** Meghan Freund[1], F. Javier deHaro-Arbano[2], Sarah Baloul[2], Abby J Ruffridge[1], Ali Torhorst[1], Andrew Q Rashoff[1], Charalambos Roussos[2], Tyler J Gibson[1], Peter Lewis[1], Sarah J Bray[2], Melissa Harrison[1] [1]University of Wisconsin-Madison, [2]University of Cambridge

The complex array of cell types that comprise an adult organism arise due to differential gene expression driven by transcription factors. The initial establishment of these gene-regulatory networks requires a specialized class of transcription factors, pioneer factors, to overcome barriers imposed by compacted chromatin and promote global changes in accessibility. This function enables pioneer factors to act at the top of gene-regulatory networks to drive developmental transitions. To better understand the properties that enable pioneer factors to access chromatin, we focus on Grainy head (Grh), an essential and highly conserved pioneer factor that drives epithelial cell fate and when mis-expressed leads to cancer. Similar to many eukaryotic transcription factors, Grh is comprised of a structured C-terminal DNA-binding domain and an extended, unstructured N-terminus, but how these domains contribute to transcription-factor binding and activity remains unknown. We used a combination of biochemistry, genomics, and quantitative imaging to identify how these structural elements influence the essential pioneering properties of Grh. We showed that full-length Grh possesses defining features of pioneer factors: it binds nucleosomes *in vitro* and drives accessibility when exogenously expressed in tissue culture. While this pioneering activity requires DNA binding, the DNA-binding domain (DBD) alone is not sufficient to promote chromatin accessibility. Single-molecule tracking revealed that Grh stably occupies the genome. This stable occupancy requires both the structured and unstructured domains of Grh, and we propose that this stable binding is an essential feature of pioneer activity. Neuroblast-specific isoforms of *Drosophila* Grh and mammalian orthologs lack the extended unstructured N-terminus. We leveraged these isoforms to further test how regions outside the DBD govern pioneering activity. Exogenous expression of these proteins revealed that they can bind and open closed chromatin. However, this activity is not as strong as with the extended N-terminus of the full-length *Drosophila* protein. To further explore this, we are using a scrambled N-terminus to disentangle the role of protein disorder versus sequence. Another feature of some pioneer factors, including Grh, is the capacity to remain bound to mitotic chromosomes, but the protein features that enable this binding and how it relates to pioneer activity remain unclear. We demonstrated that the Grh DBD is necessary and sufficient for mitotic retention, drawing a distinct contrast from pioneer activity that requires the N-terminus. Based on our assays, we propose that pioneer activity is separable from mitotic retention and requires stable chromatin occupancy driven by both structured and unstructured protein domains.

49 **Local nuclear to cytoplasmic ratio regulates H3.3 incorporation via cell cycle state during zygotic genome activation** Anusha D Bhatt[1], Madeleine G Brown[1], Aurora B Wackford[1], Yuki Shindo[1,2,3], Amanda A Amodeo[1] [1]Biological Sciences, Dartmouth College, [2]Biological sciences, UT Dallas, [3]Biological Sciences, UT Dallas

Early embryos often have relatively unstructured chromatin that lacks active and inactive domains typical of differentiated cells. In many species, these regulatory domains are established during zygotic genome activation (ZGA). In Drosophila, ZGA occurs after 13 fast, reductive, syncytial nuclear divisions during which the nuclear to cytoplasmic (N/C) ratio grows exponentially. These divisions incorporate maternally-loaded, cytoplasmic pools of histones into chromatin. Previous work found that chromatin incorporation of replication-coupled histone H3 decreases while its variant H3.3 increases in the cell cycles leading up to ZGA. In other cell types, H3.3 is associated with sites of active transcription as well as heterochromatin, suggesting a link between H3.3 incorporation and ZGA. Here, we examine the factors that contribute to H3.3 incorporation at ZGA. We identify a more rapid decrease in the nuclear availability of H3 than H3.3 over the final pre-ZGA cycles. We find that chaperone binding, not gene expression, controls incorporation patterns using H3/H3.3 chimeric proteins at the endogenous H3.3A locus. We find that the increase in H3.3 incorporation depends on the N/C ratio. Since the N/C ratio affects many parameters of embryogenesis, we further test the contributions of genomic content, zygotic transcription, and cell cycle states. We identify cell cycle regulation, but not H3 availability or transcription, as a major determinant of H3.3 incorporation. Overall, we propose a model in which local N/C ratios regulate chromatin composition via cell cycle state during ZGA.

50 **Interrogating epigenetic regulation of zygotic genome activation** Oscar M Arroyo, Mary Leatham-Jensen, Daniel McKay University of North Carolina - Chapel Hill

Differential regulation of gene expression drives specification of cell identities during development. One layer of gene control is provided by packaging of the genome into chromatin and its myriad post-translational modifications (PTMs) of histones. However, interrogating the role of histone PTMs in epigenetic gene regulation is impeded by challenging histone genetics and the complexity of the histone PTM landscape found in cells. Here, we employ two tools to interrogate the role of histone PTMs in regulating gene expression in early-stage embryos. We focus on this stage because of its relatively simple chromatin state. The zygotic genome initially lacks most histone PTMs, and PTMs are added in a stereotypical order over time until the full repertoire is established. This time-resolved elaboration of the histone PTM landscape makes the early-stage embryo an excellent model to study how histone PTMs may regulate control of gene expression. Acetylation of histone H3 lysine 18 (H3K18) and lysine 27 (H3K27) are among the first PTMs that appear during embryogenesis and are enriched at *cis*-regulatory elements for the earliest transcribed genes. By utilizing a histone gene replacement system in which a homozygous deletion of the *Drosophila* histone locus is combined with transgenic arrays encoding wild-type or non-modifiable mutant histones, we can directly test the role of individual histone residues. We find that mutation of replication-dependent H3.2K18 does not impact zygotic development. Moreover, maternal-zygotic H3.2K18 mutants are likewise viable, demonstrating that H3.2K18 is not required for development. To determine if expression of the replication-independent H3.3K18 compensates for the loss of H3.2K18 we will use H3.3K18 mutants that we have generated. Relative to H3K18, interrogating the role of H3K27 in early-stage embryos is challenging since it is required for viability. To circumvent this limitation, we developed a genetic platform that modifies the maternally deposited pool of histones which package the genome in early-stage embryos. We will use this tool to test the consequences of mutating H3K27 in isolation and in combination with H3K18 at this developmental stage. This work will elucidate the requirement of H3K18 and H3K27 in early-stage embryos, and further determine the role of histone PTMs in regulating gene expression during development.

51 **BAP/PBAP promotes GAF binding behind the replication fork** Matthew Wooten[1], Kami Ahmad[1], Steve Henikoff[2] [1]Fred Hutch Cancer Center, [2]Basic Sciences, Fred Hutch Cancer Center

Chromatin structure of genes and regulatory elements is organized by positioned nucleosomes, RNA polymerases, and localized transcription factors. These proteins organize the genome in the nucleus, regulate transcriptional activity, and control cell-type-specific gene expression. In dividing cells, DNA replication completely strips off this primary structure of chromatin, which must be reassembled behind replication forks. To track the maturation of specific chromatin features on newly synthesized DNA, we have developed nascent CUT&Tag, a chromatin profiling method that uses targeted *in* situ tagmentation to directly measure transcription factor binding on EdU-labeled nascent chromatin. Using nascent CUT&Tag, we tracked the recovery of the GAGA factor (GAF) transcription factor in *Drosophila melanogaster* Kc cells. We find that GAF is displaced from chromatin during DNA replication and recovers binding over time. While most GAF binding sites recover steady-state binding one hour after replication fork passage, a subset of GAF binding sites recover binding immediately after fork passage, while other sites recover binding much more slowly (~4 hours). Gene ontology analysis reveals that fast sites are largely found at housekeeping gene promoters, whereas slow sites are predominantly found at developmental enhancers. Fast GAF sites contain multiple strong GAF consensus DNA motifs, whereas slow sites have weak motifs. Additionally, slow sites are co-occupied with other transcription factors, suggesting that these sites require cooperative factor binding to achieve mature chromatin structure. Using chemical inhibitors, we demonstrate that the ATP-dependent chromatin remodeler BAP/PBAP (BAF) is needed for GAF to recover binding at slow sites, but not fast sites. We suggest that chromatin remodeling and cooperative transcription factor binding are necessary to enable transcription factor binding at some developmental enhancers, contributing to their regulation in dividing cells.

52 **PROPEL: a scalable model for postbaccalaureate training to promote diversity in the biomedical workforce** Jessica Allen[1], K Mark Ansel[1], Ryan Hernandez[1], Todd Nystul[2] [1]University of California, San Francisco, [2]UC San Francisco

Promoting diversity throughout the ranks of the scientific workforce is crucial for harnessing the potential of available talent and ensuring equitable access to STEM-M careers. However, entry into biomedical doctoral programs is very competitive, prioritizing applicants with prior research experience, which creates additional barriers for students who either attended colleges and universities with few opportunities to obtain research experience or who could not afford to participate in often unpaid positions. In addition, competitive doctoral programs often overlook students with non-traditional paths, such as those who discovered an interest in research later in college or switched fields. These barriers narrow the pipeline, thus contributing to a lack of diversity. Postbaccalaureate programs address this gap by offering research opportunities along with career development and graduate program application support to historically excluded groups. However, the number of these positions is restricted by available grant funding despite these same institutions regularly hiring similarly skilled scholars to research positions fully paid by individual labs. We therefore sought to develop a new model in which the career and professional development opportunities provided by a postbaccalaureate program are offered to scholars who are hired by individual labs. To facilitate this model, we developed a Matchmaking Event that provides scholars from historically marginalized groups the opportunity to meet UCSF faculty who are looking to hire research technicians and, indeed, we found this to be a highly effective recruitment tool. PROPEL, a postbaccaluareate program at UCSF with the goal of promoting diversity in the scientific workforce, builds on this success by providing scientific and career development training for these research technicians. Importantly, this model allows the program to scale dynamically according to the needs of the scientific community since scholars are hired by individual labs. At UCSF, the PROPEL program grew rapidly from six scholars in August 2020 to its current size of 116. Recently, we have initiated partnerships to establish PROPEL programs at other universities, with the goal of creating a national network of PROPEL programs. Our presentation will provide details about UCSF PROPEL, the National PROPEL initiative, and measures of both career and professional development outcomes for scholars who have participated in the program.

53 **Collaborative project-based mentorship in a virtual setting through the Genomics Education Partnership** Logan Cohen, Laura K Reed Biology, University of Alabama

With special thanks to Kellie Agrimson[2], Timothy Anderson[3], Cheryl Bardales[4], Daron Barnard[5], Indi Bose[6], Juan Carlos Martinez Cruzado[7], Don Paetkau[8], Maria Pereira[3], and Kaleb Heinrich[1]

University of Alabama[1], Saint Catherine University[2], The University of North Carolina at Pembroke[3], Louisiana State University of Alexandria[4], Worcester State University[5], West Carolina University[6], University of Puerto Rico[7], West Carolina University[8]

The Genomics Education Partnership's (GEP) Advanced Research Lab was piloted in Fall 2024 to explore and develop the pedagogical scaffolding behind comparative genomics research techniques. Led by a first-year graduate student, a group of faculty members from eight universities met weekly on Zoom to teach each other research techniques, create corresponding curriculum items, and test the new curriculum so that student feedback could be integrated during curriculum development. After attempting several mentorship structures, the group settled on a mentorship style akin to a board game night: each week had a particular challenge to which everyone in the group could contribute. The project-based collaborative approach gave all members of the mentorship group the opportunity to experience the role of both mentor and mentee relative to their experience on a given topic. Topic selection was guided by the immediate teaching needs of the group, with a focus on supporting faculty with little or no prior experience with implementing GEP curriculum in their courses. In the first three months, the group collaboratively produced seven new curriculum items which were then tested with undergraduate, graduate, and independent research students. In addition to creating and implementing new curriculum items, members were able to select from existing GEP curriculum that they wanted to implement but felt unprepared to use. The collaborative mentorship process allowed project members of all experience levels to have both mentorship support and academic freedom in their course implementations. In addition to piloting a structured, semester-long course for advanced research skills based off of Fall 2024 data, future plans include the interspersion of multi-week learning sessions for computationally intensive skills as well as a continuation of mentorship support and curriculum development for GEP instructors of all experience levels.

54 **Fly-CURE and Connecting Curriculum: Multi-Institutional Course-Based Undergraduate Research Experiences in Genetics and Beyond** Kayla Bieser[1], Jacob Kagey[2], Julie Merkle[3], Jamie Siders[4], Alysia Vrailas-Mortimer[5,6] [1]Nevada State University, [2]University of Detroit Mercy, [3]University of Evansville, [4]Ohio Northern University, [5]Biochemistry and Biophysics, Oregon State University, [6]Linus Pauling Institute, Oregon State University

The Fly-CURE is a multi-institutional course-based undergraduate research experience (CURE) centered on the genetic mapping and characterization of unknown mutations that affect cell growth and development of the *Drosophila* eye. Fly-CURE has been implemented at 28 institutions (including public, private, community colleges, and minority-serving institutions). To date, undergraduate researchers have successfully mapped and characterized 26 EMS-induced mutants, which has led to local and national scientific presentations by students, as well as twelve peer-reviewed publications with >600 undergraduate co-authors. This project has provided research exposure to greater than 1,500 undergraduate researchers within a classroom setting and student participants report significant gains in their sense of belonging to the scientific community, self-efficacy in research methods, and intent to pursue additional research opportunities. We are expanding the Fly-CURE curriculum through an NSF-funded Research Coordinated Network (RCN) to develop courses in bioinformatics, behavioral genetics, molecular biology/CRISPR, and developmental biology, which can be scaffolded with the genetics Fly-CURE modules or can be implemented as stand-alone CUREs. Through this RCN, we will increase research exposure for students across different courses and provide more opportunities for faculty to incorporate CUREs at their institutions. We are currently recruiting faculty to participate in our RCN. Faculty participants will be provided stipends for curriculum training and implementation, a social network of faculty, a community of like-minded scientists, continued scholarship opportunities, and support for tenure and promotion.

55 **Undergraduate researchers in the Fly-CURE map and characterize novel *Drosophila* mutants while experiencing increases in science self-efficacy, sense of belonging, and intent to pursue additional research experiences.** Jacob D Kagey[1], Kayla Bieser[2], Alysia Mortimer[3] [1]Biology, University of Detroit Mercy, [2]Biology, Nevada State University, [3]Department of Biochemistry and Biophysics, Oregon State University

The Fly-CURE is a consortium of faculty and students working to characterize and map novel *Drosophila* mutants within the context of an undergraduate laboratory course. The Fly-CURE project has been implemented at 30 different institutions providing authentic research experiences for over 3,000 undergraduates. The students in the Fly-CURE work to characterize and map conditional growth mutants isolated from an EMS Flp/FRT screen on chromosome 2R. To date, Fly-CURE students have characterized and mapped over 30 novel mutants and published 13 journal articles (with over 600 student co-authors). In addition to the student contribution to *Drosophila* research, we have investigated the impact of the Fly-CURE on students' attitudes towards research and their role in the scientific research community. We find that independent of faculty, institution type, or student demographic students report significant gains in all areas.

56 **Effects of Cannabinoids on Functional Ethanol Tolerance, Fertility, and Microbiome in *Drosophila melanogaster* – Insights from a C.U.R.E.** Sandra Illescas[1], Alyssa M Vidal[1], Mariano Loza-Coll[2] [1]Biology, California State University Northridge, [2]California State University Northridge

Cannabinoids have gained increasing attention for their potential health benefits, with popular compounds like CBD and THC exhibiting physiological and psychoactive effects, respectively. This study investigates the impact of CBD on functional ethanol tolerance, fertility, and microbiome in Drosophila melanogaster through a series of experiments conducted across multiple sections of an undergraduate research-based class. Flies were divided into two dietary groups: one receiving CBD-supplemented food and the other receiving standard control food. These groups were further divided into subgroups, one pre-exposed to ethanol and the other remaining naive to ethanol. To determine functional ethanol tolerance a climbing assay was conducted measuring two main metrics: climbing ability and the percentage of flies remaining active (not sedated by ethanol) over time. Our preliminary results suggest that the CBD diet significantly alters the flies ability to build a functional tolerance to ethanol. Ongoing analyses aim to assess additional factors, including fertility by evaluating eclosion rates in progeny, as well as microbial composition through whole-fly microbiome plating. These investigations provide broader insights into how CBD may impact Drosophila physiology and behavior beyond ethanol tolerance. The findings contribute valuable information on CBD's modulation of ethanol tolerance and other physiological traits, with potential implications for exploring CBD and alcohol interactions in more complex organisms, including humans. This CURE (course-based undergraduate research experience) empowers students by providing hands-on experience in scientific research, fostering critical skills in experimental design, data collection, and analysis. Such research not only advances our scientific understanding but also prepares students for future careers in the life sciences.

57 **Fun with flies: a K-12 outreach program that promotes the use of *Drosophila* as a model organism** Alexis Nagengast[1], Hemlata Mistry[2], Justin DiAngelo[3] [1]Biochemistry, Widener University, [2]Biology, Widener University, [3]Biochemistry & Molecular Biology, Penn State Berks

Outreach to the public about the use of *Drosophila* as a model organism is critical for continued support of biomedical research. Early exposure at the elementary to high school level increases the public's awareness of model organisms in general. To address this need, we have developed a collection of publically available fly mutants to build excitement and enthusiasm about *Drosophila* at different grade levels. Elementary school students view a subset of the mutants with simple magnifying glasses while middle and high school students can use dissecting microscopes. Students identify the difference between male and female wild type flies and observe flies with genetic mutations that alter wing shape, eye color, body color, body shape and muscle function. Additionally, transgenic flies that express Green Fluorescent Protein (GFP) in different body structures are displayed. We have delivered this program in various contexts including going into the elementary, middle or high schools as well as being a part of various outreach events occurring on our University campuses. Specific fly lines and grade-level appropriate talking points will be addressed.

58 **Uncovering the role of ATP synthase subunits on autophagy termination in *Drosophila*** Miriam Formica[1], Amani Al Outa[2], Siri Andresen[2], Julie Aarmo Johannessen[2], Jorrit Enserink[3], Helene Knævelsrud[2] [1]Basic medical sciences, University of Oslo, [2]University of Oslo, [3]Oslo University Hospital

Autophagy is an evolutionarily conserved degradative process essential for cellular homeostasis. Despite extensive investigation into autophagy, the molecular mechanisms that mediate autophagy termination remain poorly understood. Notably, autophagy plays a dual role in cancer, where it can either promote or prevent tumor growth. To bridge this knowledge gap, we took advantage of *Drosophila* as a model system to identify modulators of autophagy termination, aiming to discover novel therapeutic targets for cancer. Through a targeted genetic screen in GFP-mCh-Atg8a-labeled S2 cells, we identified ATP synthase subunits as critical regulators of autophagy termination. Specifically, we focused on the ATP synthase subunits *stunted* (sun) and *ATP synthase subunit F* (*ATP-synF*) due to their previously uncharacterized roles in autophagy. In the larval fat body, RNAi-mediated downregulation of *sun* and *ATP-synF* disrupted autophagy dynamics under different nutrient conditions, leading to a persistent accumulation of autophagosomes upon refeeding. Through pharmacological treatments and analysis of the GFP-mCh-Atg8a marker, we demonstrated that autophagy flux was specifically impaired in sun^{RNAi} larvae. Notably, despite this impairment, the tissue did not show accumulation of the autophagy receptor Ref(2)P. Further investigations uncovered increased phosphorylation of AMPK, a positive regulator of autophagy, in the fat body with downregulation of *sun* and *ATP-synF*. Unexpectedly, the main autophagy suppressor mTOR also exhibited higher activation in the larval fat body, indicating an aberrant regulatory feedback that disrupts autophagy termination. Collectively, our findings unveil ATP synthase subunits as pivotal regulators of autophagy shutdown, providing novel insights into the autophagy pathway and potential targets for cancer treatment.

59 **Monitoring fatty acid trafficking in follicles reveals a critical role for the triglyceride synthase DGAT1 in protecting mitochondrial integrity** Roger P White, Michael A Welte BIOLOGY, University of Rochester

During Drosophila oogenesis, lipid droplets (LDs) massively accumulate in developing follicles, particularly in nurse cells (NCs). These LDs are the main energy source of future embryo, we now report that these LDs are crucial for follicle energy metabolism as well as follicle development. We demonstrate that NC mitochondria undergo fatty acid oxidation (FAO) and that FAO is abolished in mutants for CPT1/Withered which is responsible for fatty acid (FA) import into mitochondria. CPT1 mutant ovaries have very few late-stage follicles and many mid-stage follicles show signs of degeneration. Thus, FAO is apparently crucial to produce competent eggs. By measuring mitochondrial membrane potential (MMP) using TMRE, we found that CPT1 mutant NC mitochondria exhibit lower MMP levels. This suggests that NC mitochondria rely to a substantial extent on FAO for energy production. To track how FAs traffic through follicles, we exposed follicles to fluorescently labeled FA (FLFA); the signal enriched in LDs, suggesting incoming FA are first stored as TAG in LDs. If these FAs are transferred to mitochondria for FAO, they would have to be freed via lipolysis. Indeed, in mutants for the triglyceride lipase ATGL/Brummer, MMP of NC mitochondria is reduced, suggesting FA from LDs is utilized by mitochondria. To address why FAs travel through LDs, we abolished TAG synthesis with DGAT1/midway mutants. Here, LDs synthesis is largely abolished in NCs, FLFAs accumulate in mitochondria, and mitochondria display increased mitochondrial ROS. MMP, oxygen consumption and ATP levels are all down in DGAT1 mutants, suggesting the lack of LD production negatively affects mitochondrial function. The increase in mitoROS observed in DGAT1 mutants can be rescued by ablating CPT1 function, arguing that in DGAT1 mutants, FA is excessively trafficking to mitochondria leading to metabolic stress. Since it was known that DGAT1 mutants arrest at stage 9, we hypothesize that this arrest is due to dysregulation of FA metabolism. To test this idea, we reduced the copy number of lipophorin receptors 1 and 2, which regulate FA entry into the follicle. This throttling of FA import resulted in DGAT mutants progressing past stage 9. DGAT1, CPT1 double mutants also progress past stage 9, often as far as stage 14. Since DGAT1 mutants are positive for cleaved caspase 3 activity, they may arrest due to mitochondrial induced apoptosis. Reducing the copy number of Reaper, a pro-apoptotic mitochondrial protein, led to more follicles progressing past stage 9. This work highlights the crucial role LDs have in regulating lipid metabolism; in their absence, FAs wreak havoc on mitochondria which contributes to developmental arrest and the inability of producing a viable embryo.

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Lipid Droplet Proteins ATGL and Jabba Promote *In Vivo* Collective Cell Migration in *Drosophila* Israel J Wipf[1], Sofia Gomez[1], Katherine Peregrine[2], Michelle Giedt[1], Tina Tootle[1] [1]Biology, University of Iowa, [2]University of Iowa

Lipid droplets (LDs), dynamic organelles central to lipid and energy homeostasis, have emerged as a novel factor during cell migration. *In vitro* studies found LDs promote invasion and migration in several cancer types, but the precise role of LDs during *in vivo* collective cell migration remains unknown. To address this, we used *Drosophila* border cell migration as an *in vivo* model of invasive, collective cell migration. We found that LDs within the border cells decrease in size throughout migration, suggesting lipids are being mobilized to promote migration. To test whether lipid mobilization from LDs is important for border cell migration, we analyzed migration in *Adipose Triglyceride Lipase (ATGL)* mutant flies. ATGL is a conserved LD lipase responsible for triglyceride hydrolysis and release of free fatty acids from LDs. Loss of ATGL results in both delayed migration and failed epithelial detachment (aka delamination). Loss of ATGL also results in larger LDs within the border cells compared to wildtype, suggesting ATGL-mediated lipolysis is normally active during migration. Additionally, we find another LD protein, Jabba, is critical for border cell migration. Jabba is an established LD-protein anchor, the loss of which dramatically alters LD protein composition in fly embryos. Through mutant analyses and cell-specific RNAi, we find that Jabba is required for border cell detachment and on-time migration. Surprisingly, *Jabba* mutant border cells are largely devoid of LDs, suggesting a role for Jabba in LD biogenesis and/or maintenance. This result also suggests that LD depletion in the border cells impedes migration. Future work will aim to determine how LDs function to promote border cell migration, as the stored lipids could serve as substrates for energy production or as precursors for membrane lipids and/or signaling molecules. Taken together, these results support that LDs are critical organelles for the detachment, invasion, and migration of cells *in vivo*. Our findings not only have the potential to inform how metastasizing cancer cells exploit LDs to promote their invasive behaviors, but also highlight the crucial role of LDs in the migration of non-cancerous cells during development, hinting at their broader significance in diverse migratory contexts.

61 **The role of Lamp1 in the non-cell autonomous regulation of endo-lysosomal acidification** Jonathan Handy[1], Aliza Kass[1], Satya Surabhi[1], Jingying Huang[2], Louis Romain[1], Gustavo MacIntosh[3], Andreas Jenny[1] [1]Developmental and Molecular Biology, Albert Einstein College of Medicine, [2]Albert Einstein College of Medicine, [3]Biochemistry, Biophysics and Molecular Biology and Plant Sciences Institute, Iowa State University

Alterations in lysosomal pH are linked to lower degradative capacity and thus to aberrant proteostasis and neurodegenerative diseases such as Alzheimer and Parkinson (PD). Mounting evidence suggests that LAMP proteins associate with other lysosomal membrane proteins to regulate lysosomal acidification. *Drosophila melanogaster* encodes a single *LAMP1/2* homolog, and preliminary data from our labs suggest deletion of Lamp1 leads to an accumulation of acidic vesicles in the endo-lysosomal compartment of fat bodies (FBs; akin to liver and adipose tissue), termed excessive endo-lysosomal acidification (EELA). These lysotracker-positive vesicles are not caused by activation of autophagy and show little colocalization with lysosomal markers. Endolysosomal hyperacidification phenotypes are rarely seen in disease models, with the exception of cells lacking Glucocerebrosidase 1 (GBA1) in mammals and flies. *GBA1* mutations are the cause of the lysosomal storage disease, Gaucher's Disease and are a prominent risk factor for PD. Indeed, we will present clear parallels between *Lamp1* and *Gba1b* mutants including upregulation of neuroinflammation markers and accumulation of Ubiquitin and the p62 in their brains. The suggested link between Lamp1-dependent pH regulation and PD risk factors such as *GBA1* is supported by *Lamp1* enhancing a fly PD model.

Intriguingly, individual homozygous *Lamp1* mutant FB cells of an otherwise *Lamp1* heterozygous fly, EELA is not seen. This finding suggests that *Lamp1* is either required in a tissue other than FB to prevent EELA or that *Lamp1* expression in neighboring wildtype FB cells is sufficient to rescue EELA in mutant cells. We thus have identified an unprecedented non-cell autonomous function of Lamp1 in regulation of endo-lysosomal pH. To date, we have determined that expression of *Lamp1* in FBs, neurons, and oenocytes of *Lamp1*-deficient larvae are sufficient to suppress EELA and have identified candidate components mediating Lamp1 non-cell autonomy. Additionally, we will report on a genetic screen in which we have identified at least 4 novel mutants showing an EELA phenotype.

My approach to address Lamp1 function will provide much needed insight into the regulation of endo-lysosomal acidification critical for proteostasis. It will therefore significantly advance our understanding of how dysfunction of the endo-lysosomal system underlies disease, ultimately leading to the development of novel treatment strategies to improve health span.

62 **Dissecting mechanisms that target bulk lipid transport proteins to membrane contact sites** Sarah D Neuman, Amy T Cavanagh, Arash Bashirullah University of Wisconsin-Madison

Bridge-like lipid transfer proteins (BLTPs) are a novel family of lipid transporters that engage in bulk movement of lipids at organelle membrane contact sites. Mutations in several of the BLTPs are associated with neurodegenerative and neurodevelopmental diseases. Although recent work has begun to uncover mechanisms by which BLTPs move lipids, much remains unknown about how BLTP-dependent lipid transfer function is regulated. Our work has identified subcellular targeting as a critical mechanism that regulates BLTP function. Using a combination of *in silico*, *in vitro*, and *in vivo* approaches, we found that targeting of BLTP2 (Hobbit in *Drosophila*) to endoplasmic reticulum-plasma membrane (ER-PM) contact sites is controlled by binding to specific lipid moieties. Moreover, we found that BLTP2/Hobbit enrichment at ER-PM contacts is dynamically regulated by cellular signaling pathways. Together, these findings highlight new layers of regulation that govern bulk lipid transfer at membrane contact sites.

63 **Mechanisms of cellular cannibalism in the *Drosophila* egg chamber and melanoma** Lauren Penfield, Abhinava Mishra, Morgan Smith, Denise Montell University of California, Santa Barbara

Cancer cells cannibalize other cells to survive and evade immune responses. The mechanisms that regulate cellular engulfment by tumor cells are not well understood. The small GTPase, Rac, is a key regulator of phagocytosis and its activity is often amplified in cancer. Our lab recently showed that a constitutively active form of Rac induces border cells to cannibalize other cells. Border cells are a subset of follicle cells in the *Drosophila* egg chamber. Normally, border cells carry nonmotile cells, termed polar cells, and migrate collectively between large germline nurse cells. When border cells express constitutively active Rac (Rac G12V), they do not migrate, but rather fully engulf polar cells and kill the entire germline. Death of the germline requires the engulfment receptor, Draper. However the mechanism(s) by which border cell engulfment kills the entire germline were unclear. Here, we performed live imaging to determine the sequence of events that lead to death. We compared these events to previously characterized forms of nurse cell death that occur later in development or upon starvation. Control border cells nibble on nurse cell membranes as they migrate while Rac G12V expressing border cells take larger bites, corresponding with a loss of nurse cell integrity. Rac G12V expressing border cells induce nuclear condensation and fragmentation in nurse cells adjacent to them. Then, nuclear fragmentation rapidly spreads through the germline syncytium, in a process that depends at least in part on caspase, similar to starvation-mediated death. These data support a model where excess Rac-mediated nibbling compromises nurse cell integrity, leading to caspase activation that transmits through the germline to induce death. Currently, we are testing how the oncogenic Rac mutation, P29S, affects cell engulfment in border cells and in melanoma. Rac1 P29S is the third most common driver mutation in sun-exposed melanoma, a highly cannibalistic cancer. Our preliminary work in melanoma cells suggests that Rac1 P29S cooperates with Ras and Raf oncogenic mutations to enhance cannibalism of T cells. We are investigating how activating mutations in Rac, Ras, and Raf contribute to cannibalism and migration behavior in the border cell model. Together this work reveals how activation of Rac in a small subset of follicle cells induces germline death and has the potential to reveal how oncogenic mutations contribute to cancer cell cannibalism.

64 **Ceramide and its metabolites regulate the assembly and release of extracellular microcarriers** Mark Wainwright[1], Amy Cording[2], Abigail Pavey[2], Soutiam Goodarzi[2], Bhavna Verma[2], Adam Wells[2], Jade Oh[2], Lewis Blincowe[2], Clive Wilson[2] [1]Physiology, Anatomy & Genetics, University of Oxford, [2]University of Oxford

Intercellular signalling not only involves soluble signals, but can also be mediated by lipophilic molecules. For example, breast milk lipid droplets carry metabolites and signalling molecules to the new-born, but the mechanisms regulating droplet assembly remain poorly understood. We have previously shown that the lumen of the *Drosophila* male accessory gland (AG) contains many ellipsoid, neutral lipid-containing structures, termed microcarriers, which carry the archetypal reproductive signalling protein, Sex Peptide (SP), at their surface and transfer it to females within seminal fluid. Surprisingly, SP is required for the proper formation of microcarriers, preventing their fusion into large lipidic droplets.

To identify other microcarrier regulators, we knocked down genes that are highly expressed in the male AG. RNAi knockdown or a hypomorphic mutant of *Ugt50B3*, the fly homologue of human ceramide galactosyl transferase UGT8, induces a dense network of neutral lipid-containing extracellular projections at the apical surface of the major epithelial cell type in the AG, main cells. These projections carry SP-GFP and some link to microcarriers, which are often abnormally clustered in the AG lumen. We show that galactose must be transported into the ER to permit normal microcarrier assembly and that *UGT50B3* knockdown males are infertile.

Interestingly, blocking *de novo* ceramide synthesis using RNAis targeting *schlank* and *ifc* (infertile crescent), or inhibiting ceramide transfer from ER to Golgi (*cert* knockdown), or ceramide phosphoethanolamine synthesis (*Cpes* knockdown) completely disrupts microcarrier assembly and stability; neutral lipid is not condensed into ellipsoid structures and SP is separated from the diffuse lipid remaining within the AG lumen. Knockdown of the secreted ER protein MANF (Mesencephalic Astrocyte-Derived Neurotrophic Factor), which binds to ceramide derivatives, produces a similar phenotype that does not appear to be associated with ER stress, which MANF is known to modulate. Knockdown flies have low fertility, but absence of ceramide metabolites, rather than defective microcarrier structure, appears to be the primary cause. We conclude that ceramide and its metabolites are critical for microcarrier formation, while galactosylceramide, the most abundant cerebroside in human breast milk fat droplets and a critical player in separating membranes during myelination, is essential for microcarrier release from secreting cells.

65 **Apocrine secretion: a novel non-canonical and non-vesicular transport and secretory mechanism discovered in the salivary glands of *Drosophila*** Denisa Beňová-Liszeková[1], Milan Beňo[2], Lucia Mentelová[3], Klaudia Babišová[2], Adam Chorovský[2], Bruce A Chase[4,5], Robert Farkas[6] [1]Laboratory of Developmental Genetics, Institute of Experimental Endocrinology, Slovak Academy of Sciences, [2]Laboratory of Developmental Genetics, Institute of Experimental Endocrinology, Biomedical Research Center, Slovak Academy of Sciences, [3]Department of Genetics, Comenius University, [4]Department of Biology, University of Nebraska, [5]Departments of Neurology & Data Analytics, Northshore University Health System, [6]Laboratory of Developmental Genetics, Institute of Experimental Endocrinology, Biomedical Research Center, Slovak Academy Sciences

Apocrine secretion (AS) is a newly characterized non-canonical and non-vesicular cellular signaling pathway that has been re-defined with respect to its use in the *en mass* transport and externalization of proteins and cytoplasmic material. It uses a strikingly different mechanism of transport and secretion than that used in the more widely studied exocytosis. Focused research studying AS in the salivary glands (SGs) of *Drosophila melanogaster* has begun to elucidate its molecular and genetic determinants. In the SGs, massive AS takes place in the late prepupal period, substantially after after Sgs-glue secretion. This secretion serves to deliver a fully immunocompetent exuvial fluid that bathes the metamorphosing pupae during pupation. Scrutinizing 32 *Drosophila* species and two more distantly related fly species for the presence of AS in the prepupal SGs revealed that, in contrast to the production and exocytosis of Sgs-glue, AS is a phylogenetically conserved function in all fly SGs. In the course of this work we identified two new organelles that are used in AS: collector sacculae and apocrine channels. Both of these are derivatives of apical microvilli that deeply invaginate into SG cells during AS. In-depth proteomic analyses identified more than 1500 proteins present in the secretion. Strikingly, these derive from microsomal, membraneous, cytoskeletal, mitochondrial, ER- and Golgi-, nuclear and even nucleolar origins. Comparison of the *Drosophila* SG and various human-AS proteomes revealed that their ontological categories are evolutionarily strongly conserved from flies to man, even though these proteins have a range of different biological/molecular functions and sub-cellular origins. We initiated a genomic screen in *Drosophila* to systematically search for AS effectors. This identified more than 20 loci that when manipulated either prevent, up-regulate or precociously initiate AS, or alter the transport of a cargo. Since many of these loci are previously uncharacterized *CG*-genes - this work uncovered their function - some of the genes used in the regulation of AS appear to provide novel functions. We will present data on the cellular mechanism of AS and the phenotypes of *CG* genes that have identified key elements of the molecular pathway used by the AS process.

66 **Evolution in molecular interactions underlies interspecies incompatibility of a cytoplasmic cell fate determinant** Emily L Rivard[1], John R Srouji[1], Cassandra G Extavour[1,2,3] [1]Molecular and Cellular Biology, Harvard University, [2]Organismic and Evolutionary Biology, Harvard University, [3]Howard Hughes Medical Institute

Novel and rapidly evolving genes can integrate into conserved gene networks and play critical roles in development. Understanding how sequence variation across the orthologs of such genes influences interactions with other molecules requires investigation at the level of protein function. Here, we examine this molecular scale to determine how sequence evolution in *oskar,* a gene required for germ cell specification and embryonic patterning in fruit flies, has led to functional divergence between orthologs from *Drosophila melanogaster* and *Drosophila virilis*. We generated chimeric versions of *oskar* by interchanging Oskar protein's four functional domains from each species in sixteen combinations. We expressed these chimeric *oskar* sequences in *D. melanogaster* and quantified their ability to recruit germ line and abdominal patterning determinants (germ plasm) to the posterior end of the oocyte and early embryo. We identified chimeras containing specific portions of *D. virilis* Oskar that could not maintain germ plasm at the posterior well enough to generate pole cells but were sufficient to specify the anterior-posterior axis of *D. melanogaster oskar* null embryos. We also found regions in the *D. virilis* Oskar sequence with dominant-negative effects on *D. melanogaster* Oskar's ability to localize germ plasm mRNA, resulting in severe axial patterning defects. Furthermore, our data suggest that the structure-function map of Oskar may not be as modular as previously believed, as multiple domains impact Oskar's ability to perform key roles like mRNA-binding. Our evolutionary approach provides novel *in vivo* evidence of the molecular action of Oskar's functional domains. We propose new hypotheses about how the posterior localization of Oskar is maintained. Our findings also offer insight into how efficiently germ plasm components must be localized for different developmental processes to progress successfully. This study underscores the value of investigating molecular interactions to bridge genome evolution with phenotypic variation at higher scales.

67 **Single-cell transcriptomes reveal largely sex-coupled evolutionary changes of sexual circuits in *Drosophila*** Dawn S Chen, Yun Ding Biology, University of Pennsylvania

Sexual dimorphism in neural circuits reflects the divergent behavioral strategies employed by different sexes to achieve reproductive fitness. However, emerging data in vertebrate and invertebrate species also find substantial similarity between sexes in sexual circuit anatomy and cell types. How sexual circuits evolve under sex-specific fitness consequences and sex-shared circuit architecture remains largely unknown. In *Drosophila melanogaster* and closely related species, the *fruitless* (*fru*)-expressing neural circuit is essential for sexual behaviors, including rapidly evolving male courtship behaviors, and is thus expected to be the "battleground" of sexual conflict and hotspot of sexual dimorphism in the nervous system. Here, we leverage the genetic accessibility of the adult *fru* sexual circuit and the cell type resolution of single-cell transcriptomics to understand the forces that shape cell type and gene expression evolution in sexual circuits between *D. melanogaster* and *D. yakuba.* Both sexes share similar *fru* circuit neuroanatomy, and we identified clear homologs of transcriptomically defined cell types ("clusters") between sexes and species. Sexual dimorphism in cluster size and gene expression is limited and mostly species-specific, with comparable levels of female- *versus* male-bias. In contrast, species divergence in cluster size and gene expression are prevalent, but largely coupled between sexes. This result is consistent with the largely sex-shared genome imposing a constraint on sex-specific gene expression evolution. Finally, we sought to understand the role of sex-specific *fru* isoforms, which are also essential for male sexual behaviors, on shaping *fru* circuit evolution. Surprisingly, loss of *fru* causes drastic and pervasive effects on cluster size and gene expression, far exceeding the extent of sexual dimorphism in control animals. The affected genes are overrepresented among those with sex-specific or species divergent gene expression. In summary, despite the importance of sexual dimorphism in behavior, we find that males and females are largely coupled in evolutionary trajectory, and suggest that an adult sexual circuit with selective dimorphic sites among a largely sex-shared architecture enables the orchestration of sex-specific behaviors while retaining evolvability.

68 **TF-High-Evolutionary: In Vivo Mutagenesis of Gene Regulatory Networks for the Study of the Genetics and Evolution of the Drosophila Regulatory Genome** Xueying Li[1,2], Vani Srinivasan[2], Ian Laiker[3], Natalia Misunou[2], Nicolás Frankel[3], Luisa F. Pallares[4], Justin Crocker[2] [1]Beijing Normal University, [2]European Molecular Biology Laboratory, [3]Instituto de Fisiología, Biología Molecular y Neurociencias (IFIBYNE), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) y Universidad de Buenos Aires (UBA), [4]Friedrich Miescher Laboratory, Max Planck Society

Understanding the evolutionary potential of mutations in gene regulatory networks is essential to furthering the study of evolution and development. However, in multicellular systems, genetic manipulation of regulatory networks in a targeted and high-throughput way remains challenging. In this study, we designed TF-High-Evolutionary (HighEvo), a transcription factor (TF) fused with a base editor (activation-induced deaminase), to continuously induce germline mutations at TF-binding sites across regulatory networks in Drosophila. Populations of flies expressing TF-HighEvo in their germlines accumulated mutations at rates an order of magnitude higher than natural populations. Importantly, these mutations accumulated around the targeted TF-binding sites across the genome, leading to distinct morphological phenotypes consistent with the developmental roles of the tagged TFs. As such, this TF-HighEvo method allows the interrogation of the mutational space of gene regulatory networks at scale and can serve as a powerful reagent for experimental evolution and genetic screens focused on the regulatory genome.

69 **Trichome-specific polyploidy underlies extreme specializations of a genital novelty in *D. eugracilis*** Gavin Rice[1], Shyama Nandakumar[2], Mark Rebeiz[3] [1]University of Pittsburgh, [2]Department of Cell Biology, University of Pittsburgh School of Medicine, [3]Biological Sciences, University of Pittsburgh

Many novel traits have been hypothesized to be formed through genetic network co-option, where a genetic network becomes activated in a new part of the body plan. Even though genetic network co-option has been implicated many times, few cases address how these networks become rewired to produce a novelty's unique characteristics.

The rapidly evolving genitalia of *Drosophila* provide a powerful system for studying the developmental basis of morphological novelty. We investigated how the phallus of *Drosophila eugracilis* gained over 150 differently sized projections. Developmental tracking of cellular morphology uncovered that these projections are unicellular. This was surprising as these unicellular projections vary 20-fold in size. Our previous work showed that the main transcription factor for the larval trichome (unicellular hairs) genetic network, Shavenbaby, and 14 of its known downstream targets were co-opted to the *D. eugracilis* phallus.

Although modulation of the trichome network affects the morphology of unicellular projections, it does not seem to account for the large cellular size seen in *D. eugracilis*. We find that large unicellular projections are produced from cells with large nuclei, indicating that these cells have undergone localized endoreplication to generate polyploid trichome nuclei. These large cells also express the polyploidy-inducing gene *fizzy-related* during their development. Furthermore, activation of the larval trichome genetic network in the phallus of *Drosophila melanogaster*, which naturally lacks these unicellular projections, induces only small unicellular projections. At the same time, the disruption of *shavenbaby* in *D. eugracilis* reduces unicellular projection height but shows no obvious effect on overall cellular size. This suggests that a second independent genetic network was recruited to the *D. eugracilis* unicellular projections to induce their most exaggerated phenotypes. Our work shows how traits that arise through gene regulatory network co-option may initially evolve as a simple copy and subsequently gain unique attributes through the recruitment of additional genetic networks.

70 **The ribosomal locus mediates a single-locus hybrid incompatibility in *Drosophila*** Emiliano Marti[1], Christina A Muirhead[1,2], Lori Wright[1], Daven C Presgraves[3] [1]Biology, University of Rochester, [2]Ronin Institute, [3]University of Rochester

The species of the *Drosophila simulans* clade—*D. simulans*, *D. mauritiana* and *D. sechellia*— diverged ~240,000 years ago and serve as a model system for speciation research. Hybrid male sterility factors accumulate faster than other kinds of hybrid incompatibilities; however, even between these young species, a small number of strong hybrid lethality factors has evolved. One of these, *hybrid lethal on the X* (*hlx*), maps to the pericentric heterochromatin of the X chromosome of *D. mauritiana* (mau) and causes male lethality in the genetic background of its sibling species. Despite the strong lethality associated with *hlx*^{mau}, we have identified "escapers"— *i.e.,* surviving *hlx*^{mau} males with an otherwise lethal genotype. These escaper males invariably show delayed development, reduced sizes, short/thin bristles, and abdominal etching. These phenotypes are reminiscent of *bobbed* mutations in *D. melanogaster*, which correspond to deletions in the rDNA locus. *hlx*^{mau} males also show a drastic derepression of two non-LTR retroelements, *R1* and *R2*, that insert site-specifically into the *28S* subunit of rRNA genes, rendering them non-functional. The rDNA locus comprises hundreds of tandemly arrayed rRNA genes, and >120 copies are required to sustain wildtype development. We introgressed different *hlx* alleles from a panel of *D. mauritiana* X chromosomes into *D. simulans* and observed a bimodal distribution of phenotypes— lethality and complete rescue— suggesting that *hlx* segregates in *D. mauritiana*. qPCR assays revealed a perfect association: lethal *hlx*^{mau} have low rDNA copy numbers. Together these findings imply that *hlx*^{mau}-mediated lethality results from severe ribosomal insufficiency. Like most *Drosophila* species, *D. mauritiana* has X- and Y-linked rDNA loci, whereas *D. simulans* and *D. sechellia* secondarily lost their Y-linked rDNA locus. We hypothesize that the Y-linked rDNA of *D. mauritiana* allows smaller X-linked rDNA arrays to segregate and, conversely, loss of the Y-linked rDNA in its sibling species was compensated by the expansion of X-linked rDNA. Consequently, *hlx*-mediated lethality in hybrids arises from exposing otherwise recessive rDNA-deficient loci in *D. simulans* and *D. sechellia*. Hybrid incompatibilities almost always involve epistatic interactions between two or more loci. Our findings, however, reveal a different scenario— a species-specific chromosomal rearrangement (loss of Y-linked rDNA) can lead to the evolution of a single-locus hybrid incompatibility.

71 **Pervasive transcriptome-wide parallel adaptation in *D. melanogaster* and *D. simulans* male reproductive tracts reveals fine-tuning of intraspecific gene expression variation by natural selection** Tiezheng Fan, David Begun Department of Evolution and Ecology, University of California, Davis

Convergent evolution occurs when different species evolve similar phenotypes in response to similar selective pressures, and provides compelling evidence for adaptation. While stabilizing selection is likely an important process leading to conserved patterns of gene expression between species, the role of selection in driving expression adaptation and divergence amongst populations is less clear. *D. melanogaster*, and its sister species, *D. simulans*, have been focal species in several comparative studies. Latitudinal clines constitute a model system for studying adaptation in *Drosophila*, and multiple species exhibit clinal phenotypic differentiation for traits such as body size and wing size. Gene expression parallelism for latitudinally differentially expressed genes in whole male transcriptomes has been reported between North American *D. melanogaster* and *D. simulans*. However, whether selection shapes transcriptome-wide patterns of gene expression at the tissue level in these species remains unknown. Here, we further investigate transcriptomes of North American flies with a focus on the male reproductive tracts. For both species we sampled accessory glands and testis from two locations, one high latitude, Fairfield, Maine, and one low latitude, Panama City, Panama. We observed a striking similarity in the directionality and magnitude of change in transcriptome-wide expression between the species in the accessory glands but not in the testis, suggesting that selection has fine-tuned accessory gland transcript abundance in response to latitudinal pressures in these two species. To investigate if selection may shape accessory gland transcriptomes on a larger-than-gene scale, we estimated means of log-fold-change values in multi-gene windows of different scales. These analyses revealed that the two species exhibit highly correlated fluctuations of high vs. low latitude expression differences on an at least 50 kb scale. However, these larger scale fluctuations cannot be entirely explained by gene level parallelism. This suggests that extensive parallel expression adaptation to high vs. low latitude environments is acting on multiple scales, from genes to chromosomal regions.

72 **Coevolution between a DNA satellite and Topoisomerase II triggers an inter-species incompatibility** Cara L Brand, Mia T Levine Biology, University of Pennsylvania

Inter-species incompatibilities do not arise as a direct result of natural selection. Instead, they emerge as an incidental byproduct of lineage-specific evolution and manifest in the unique genomic background of interspecies hybrids. Intra-genomic coevolution between rapidly evolving species-specific satellite DNA and their interacting proteins is thought to be a powerful force driving inter-species incompatibilities. While there is growing evidence for this model, there are very few cases in which both incompatible factors have been identified and functionally characterized, hindering our ability to understand the gene functions and molecular mechanisms that trigger inter-species incompatibilities. To reveal the diverged molecular machinery and cellular pathways underlying species incompatibles, we experimentally probed coevolution between the *Drosophila melanogaster*-specific *359bp* satellite array and the adaptively evolving protein, Topoisomerase II (Top2). Previous work demonstrated that *D. melanogaster* Top2 is required to resolve DNA entanglements at the *359bp* array and that Top2 accumulates at *359bp* in the early embryo. This DNA repair function, combined with a signature of adaptive evolution, raises the possibility that the *D. melanogaster*-specific *359bp* satellite requires a *D. melanogaster*-specific version of Top2 to resolve DNA entanglements. To test this, we engineered *D. melanogaster* female flies that maternally deposit a diverged *D. simulans* version of Top2 into the embryo. We find that maternal Top2 from *D. simulans* triggers early embryonic lethality. Using satellite-specific FISH probes, we demonstrate that embryo lethality is triggered by missegregation of the *359bp* array. This finding suggests that the *D. simulans* version of Top2 is incompatible with *D. melanogaster*-specific *359bp* satellite. Consistent with this finding, we rescue embryogenesis by deleting the *359bp* array, demonstrating that this incompatibility is specific to the *D. simulans*version of Top2 and the *D. melanogaster*-specific *359bp* array. To determine how the D. simulans version of Top2 compromises *359bp* integrity, we are characterizing embryonic viability in the presence of chimeric *D. melanogaster-D. simulans* Top2 proteins. These experiments will reveal the mechanistic basis of this interspecies incompatibility. Intriguingly, this incompatibility is reminiscent of a long-studied reproductive barrier between *D. melanogaster* and *D. simulans*, raising the possibility that coevolution between *359bp* and Top2 underlies F_1 hybrid lethality. Our study highlights how rapid satellite turnover shapes genome integrity and provides novel mechanistic insights into how satellite evolution triggers reproductive barriers between species.

73 **Polymorphic 3D genome architecture mediated by transposable elements** Harsh Girish Shukla, Grace Yuh Chwen Lee Ecology and Evolutionary Biology, University of California, Irvine

The intricate three-dimensional (3D) folding of genetic material within the tiny space of a cell nucleus plays a crucial role in shaping genome regulation. However, how 3D genome structure varies between individuals and influences genome function and evolution remains poorly understood. In this study, we investigate the role of transposable elements (TEs) in shaping polymorphic 3D genome architecture. TEs are genomic parasites that selfishly replicate at the expense of host fitness. To combat their selfish behavior, hosts typically silence TEs through the enrichment of repressive epigenetic marks, such as H3K9me2/3, which often "spread" into adjacent regions, turning TE local regions into islands of heterochromatin within euchromatin. Previous studies have shown that euchromatic H3K9me2/3-enriched regions, including TEs, can spatially interact with pericentromeric heterochromatin (PCH) through condensate formation, despite large linear distances between them. Interestingly, despite the prevalence of TEs in the Drosophila genome, most insertions are found in only a few individuals. Taken together, we hypothesize that the presence/absence polymorphism of TEs drives polymorphic 3D nuclear structures through TE-PCH spatial interactions.

To test our hypothesis, we performed Hi-C for two inbred D. melanogaster wildtype strains with distinct TE insertion profiles. We generated reference-quality genome assemblies of these strains using PacBio HiFi, achieving superior PCH coverage compared to the current Drosophila reference, and developed a novel pipeline to compare 3D proximity to PCH between homologous alleles. Supporting our hypothesis, we found significantly more frequent 3D interactions with PCH in TE euchromatic neighborhoods when the TE was present compared to TE-free homologous alleles. TEs spatially interacting with PCH are enriched with higher levels of H3K9me3 and are more likely to be RNA-based TEs. Surprisingly, even TEs close to telomeres show a similar propensity to interact with PCH compared to TE-free homologs. Importantly, we found that TEs involved in 3D interactions with PCH perturb the expression of TE-adjacent genes and have lower population frequencies than other TEs, suggesting they are more strongly selected against. Our study reveals a previously unknown mechanism by which these selfish genetic elements influence both the function and evolution of the host genome through generating polymorphic 3D genome organization.

74 **Integrated single-embryo transcriptomics and metabolomics reveal dynamic molecular programs in early *Drosophila* development.** Eduardo Perez-Mojica[1], Zachary Madaj[2], Christine Isaguirre[3], Kin Lau[2], Joe Roy[1], Ryan Sheldon[3], Adelheid Lempradl[1] [1]Metabolism and Nutritional Programming, Van Andel Institute, [2]Bioinformatics and Biostatistics Core, Van Andel Institute, [3]Mass Spectrometry Core, Van Andel Institute

Embryogenesis lays the foundation for animal development, marked by a crucial transition from maternal factor reliance to zygotic control. While transcriptional events during early development are well-characterized, concurrent metabolic processes remain poorly understood, particularly during early embryo stages. These metabolic events are critical for cell fate determination and tissue formation but have been challenging to study due to technical limitations arising from the minimal material available. To overcome these challenges, we present a novel single-embryo methodology for the simultaneous analysis of the metabolome and transcriptome in *Drosophila melanogaster* embryos with high temporal resolution. By integrating RNA-sequencing and metabolomics data from individual embryos, we establish time-resolved developmental trajectories (pseudo-time) and transcriptional signatures, enabling robust correlation of metabolic and transcriptional programs.

Our method reveals dynamic patterns in both transcripts and metabolites, including key metabolic changes preceding zygotic genome activation. For instance, deoxyribonucleotide triphosphate (dNTP) levels exhibit temporal dynamics aligned with cell division cycles, showing a significant decrease at the onset of zygotic transcription. The dataset comprises 7,367 transcripts and 81 metabolites per embryo, providing unprecedented insights into developmental processes. Additionally, we identify novel sex-specific transcriptional differences without requiring genotyping, enhancing reproducibility across laboratories. This comprehensive approach has unveiled previously unrecognized connections between metabolic and transcriptional landscapes, such as the interplay between deoxyribonucleotide metabolism and developmental timing.

In summary, our single-embryo methodology delivers a high-resolution view of early embryonic development, combining metabolomics and transcriptomics to map integrated programs with unparalleled sensitivity. This robust tool opens new avenues for understanding the roles of developmental and metabolic genes, sex-specific embryonic reprogramming, and the complex molecular dynamics of early development.

75 **Role of kidney Coenzyme A biosynthesis in systematic metabolic control and maintenance of tissue homeostasis in high-turnover tissues** Ting Miao[1], Kerui Huang[2], Ying Liu[2], Christian Dibble[3], Norbert Perrimon[1] [1]Harvard Medical School, [2]Genetics, Harvard Medical School, [3]Beth Israel Deaconess Medical Center

An enormous amount of groundbreaking work has gone into understanding the sources and regulation of acyl groups and acyl-CoAs in the synthesis, handling, and degradation of lipids under both physiological and pathological conditions. However, comparatively little attention has been given to how Coenzyme A (CoA) availability, a critical cofactor in lipid metabolism, is synchronized with CoA-dependent metabolic pathways. Enzymes and metabolites within the CoA biosynthesis pathway exhibit evolutionary conservation. CoA is *de novo* synthesized from pantothenate by Pantothenate Kinases 1-3 (PANK1-3), with subsequent enzymatic reactions yielding CoA. Recent research identified PANK4 as a rate-limiting suppressor targeting the third intermediate in CoA synthesis by dephosphorylation. However, the *in vivo* function of PANK4 is still elusive, and the Drosophila PANK4 orthologue, CG5828 (dPANK4), has not been characterized at all. Our snRNA-Seq analysis revealed that *dPANK4* is highly expressed in the principal cells of Malpighian tubules (MTs), analogous to mammalian proximal tubules. In vivo isotope tracing and targeted metabolomics following MT principal cell-specific dPANK4 knockdown showed elevated CoA and acyl-CoA levels, suggesting a conserved role for dPANK4 in CoA metabolism. Given the importance of CoA biosynthesis in lipid metabolism, we explored whether dPANK4 impacts body fat accumulation. Remarkably, whole-body overexpression of dPANK4 significantly reduced triglyceride (TAG) levels, whereas dPANK4 knockdown increased TAG levels, consistent with mammalian cell observations. This effect on fat accumulation was further amplified with MT-specific dPANK4 knockdown. Lipidomics analysis revealed that MT dPANK4 affects not only glycerol lipids but also glycerophospholipids, sphingolipids, and sterol lipids, highlighting its broader influence on the lipidome. Beyond lipid metabolism, our findings indicate that MT CoA synthesis plays a role in regulating intestinal stem cell (ISC) proliferation. Increased phospho-histone 3 (pH3) signals, a marker of mitosis, were observed in the midgut following activation of CoA synthesis in the MT via dPANK4 knockdown. Conversely, inhibition of CoA synthesis in MTs by knockdown of fumble (Drosophila PANK1-3), the rate-limiting enzyme in CoA biosynthesis, slowed ISC proliferation and tumor progression in a fly gut tumor model. These findings underscore the critical role of kidney CoA biosynthesis in systemic metabolic regulation and maintenance of tissue homeostasis in high-turnover tissues.

76 ***In vivo* bioorthogonal tagging and tracing of branched-chain amino acids and their metabolites** Sebastian Sorge[1], Victor Girard[2], Vanessa Nunes[2], Paul C. Driscoll[2], Alex P. Gould[2] [1]The Francis Crick Institute, London, [2]The Francis Crick Institute

To understand metabolism in the context of a living animal, we need to be able to monitor metabolite transport from one cell/tissue type to another. This has been a long-standing challenge in the field because *in vivo* methods to tag or label nutrients and metabolites in a cell-type specific manner are currently lacking. To overcome this limitation for amino acids, we have developed a genetically-encoded method called *conditional activation of amino acids by stereoinversion* (CAAS). CAAS utilizes conditional expression of microbial enzymes that convert metabolically inactive D-amino acids into their bioactive L-steroisomers. As proof-of-principle in *Drosophila*, we used GAL4/UAS to express LIRase, an epimerase specific for the essential branched-chain amino acids (BCAAs) leucine and isoleucine. LIRase, and the CAAS methodology in general, can be utilized in either bulk or tracing modes depending upon the primary objective. In bulk mode, we show that enterocyte-specific expression of LIRase is sufficient to rescue lethality and to promote normal growth and development on a diet where L-leucine is replaced with D-leucine. Similar findings using LIRase were also obtained in mice. Using LIRase in tracer mode in *Drosophila*, we devised a strategy for cell-type specific isotope labelling of a small fraction of the total L-leucine. This tracer mode enabled determination of the relative contributions of individual tissues such as the gut and fat body to the hemolymph or CNS pools of leucine. A similar interorgan tracing methodology can also be used to calculate the hitherto unknown contributions of L-leucine carbons from remote tissues to the lipidome of the CNS. This study illustrates that CAAS is a powerful technology with wide applications in biomedical research for tracing amino-acid transport and catabolism from cell-to-cell.

77 **Adipocyte heterogeneity regulated by the Bithorax Complex and Wnt/Wingless signaling crosstalk in *Drosophila*** Rajitha Udakara Sampath Hemba-Waduge[1], Mengmeng Liu[1], Xiao Li[2], Jasmine L Sun[1], Elisabeth A Budslick[1], Sarah E Bondos[3], Jun-Yuan Ji[1] [1]Biochemistry and Molecular Biology, Tulane University School of Medicine, [2]Lewis-Sigler Institute of Integrative Genomics, [3]Department of Medical Physiology, Texas A&M University Health Science Center

Adipocytes in different body regions perform distinct functions and are regulated by unique mechanisms, with varying metabolic profiles essential for lipid metabolism and energy homeostasis. Disruptions in these processes are linked to disease conditions such as obesity, diabetes, cardiovascular diseases, and cancer. Notably, the specific regions of excessive fat accumulation can pose distinct health vulnerabilities where visceral fat around internal organs causes a higher risk compared to subcutaneous fat in the lower body. However, the signaling pathways and developmental mechanisms underlying this regional adipose tissue heterogeneity are poorly understood. In this study, we demonstrate that interactions between Wnt/Wingless signaling and *Bithorax Complex* (*BX-C*) genes – specifically *abdominal A* (*abd-A*) and *Abdominal B* (*Abd-B*) – drive regional differences in *Drosophila* larval adipocytes. Abdominal adipocytes, expressing elevated levels of *abd-A* and *Abd-B*, differ significantly from thoracic adipocytes, with activated Wnt signaling amplifying these differences. Depleting *abd-A* and *Abd-B* in adipocytes reduces fat accumulation, delays larval-pupal transition, and leads to pupal lethality, contrasting with the effects of attenuated Wnt signaling on lipid mobilization. Importantly, Abd-A and Abd-B play a permissive role in modulating both Wnt-activated and Wnt-repressed gene expression in larval adipocytes, especially in abdominal adipocytes, where these genes are endogenously expressed. The presence of Abd-A and Abd-B in abdominal adipocytes enables them to respond to Wnt signaling by promoting lipid mobilization and repressing lipogenesis. In contrast, adipocytes lacking *abd-A* and *Abd-B* expression – whether thoracic or occasionally abdominal – fail to activate or repress Wnt target genes, rendering these adipocytes unresponsive to Wnt signaling-induced lipid mobilization. Taken together, Wnt signaling stimulates *abd-A* and *Abd-B* transcription, creating a feedforward loop that reinforces the interaction between Wnt signaling and *BX-C* genes. These findings elucidate how the crosstalk between cell-autonomous *BX-C* gene expression and Wnt signaling defines adipocyte heterogeneity in *Drosophila* larvae.

78 **Lactate and glycerol-3-phosphate metabolism cooperatively regulate larval growth in a tissue nonautonomous manner** Madhulika Rai[1], Shefali Shefali[2], Sarah Carter[3], Hongde Li[4], Geetanjali Chawla[5], Robert Policastro[6], Gabriel Zentner[6], Jason Tennessen[6] [1]Biology, Indiana University Bloomington, [2]Biology, Indiana University, [3]Biology, University of Michigan, [4]Investigator at Hangzhou Institute of Medicine, CAS, [5]Shiv Nadar Institute of Eminence, [6]Indiana University

Drosophila larval development requires the rapid conversion of nutrients into biomass. In response to these biosynthetic demands, larval metabolism exhibits the hallmark features of aerobic glycolysis, a metabolic program ideally suited to synthesize macromolecules from carbohydrates. Previous studies from our lab have demonstrated that the enzymes Lactate Dehydrogenase (LDH) and Glycerol-3-phosphate dehydrogenase (GPDH1) are essential for cooperatively maintaining the larval glycolytic program. Although disruption of either enzyme has minimal effect on larval growth, simultaneous loss of both enzymes results in a synthetic lethality and aberrant carbohydrate metabolism. These findings, however, are based on studying loss of *Ldh* and *Gpdh1* in the whole body and raise the question of how these two enzymes coordinate larval metabolism across multiple tissues. To address this question, we have used RNAi to determine how tissue-specific depletion of *Ldh* and *Gpdh1* affects larval growth and metabolism. Our results demonstrate that loss of *Ldh* within either the fat body or muscle of *Gpdh1* mutants lead to systemic growth defects in larvae. Hence, *Ldh* and *Gpdh1* can influence larval growth and metabolism in a cell nonautonomous manner, indicating that the cooperative activity of these two enzymes within individual tissues can induce systemic signals that coordinate intercellular metabolic states with growth of the entire organism. To find these systemic signals, we performed transcriptomic analysis and interestingly, found that the cytokine UPD3 gets upregulated in response to changes in LDH and GPDH1 activity. Further, loss of *Upd3* in *Ldh; Gpdh1* double mutant larvae showed milder growth defects in comparison to *Ldh; Gpdh1* double mutant. This suggests that *Upd3* is a key systemic signal coordinating whole larval growth with tissue-specific levels of LDH and GPDH1. Overall, our findings hint at a mechanism that coordinates larval growth with the rate of glycolytic flux in individual tissues.

79 **Rapamycin Fly Cell Atlas reveals pro-longevity impact across the whole organism at cellular resolution** Tzu-Chiao Lu[1], Chung-I Liang[1,2], Bo Sun[1], Ao-Lin Hsu[2], Yanyan Qi[1], Hongjie Li[1] [1]Baylor College of Medicine, [2]National Yang Ming Chiao Tung University

Rapamycin extends lifespan across multiple species, yet its effects on individual cell types remain unclear. Here, using Drosophila melanogaster, we present the Rapamycin Fly Cell Atlas (Rapa-FCA), a comprehensive single-nucleus transcriptomic profile of the entire organism. This atlas identifies over 200 cell types across head and body tissues, both sexes, and young and old animals. We find that rapamycin predominantly affects the transcriptomes of body cells, decelerating aging trajectories in several somatic and reproductive cell types, while inducing minimal changes in the nervous system. Beyond transcriptomic alterations, rapamycin attenuates aging-associated changes in nuclear number and cell-type identity. By comparing rapamycin-regulated aging features, we establish geroprotection scores for each cell type and identify those with high rapamycin-induced geroprotection. Our data provide a valuable resource for understanding pro-longevity mechanisms in complex organisms.

80 **Glycolysis is required in the female germline for stem cell maintenance and egg chamber growth, but not for mitotic proliferation, meiotic entry, or oocyte specification** Emily M Wessel[1,2], Daniela Drummond-Barbosa[1,2] [1]Genetics, University of Wisconsin-Madison, [2]Morgridge Institute for Research

Adult stem cell lineages are vital for tissue maintenance and are responsive to environmental cues such as nutritional availability. However, their intrinsic metabolic requirements in living organisms with complex tissues made of multiple cell types remains unexplored. In the *Drosophila* ovary, two very different stem cell lineages - derived from germline stem cells and somatic follicle stem cells - closely interact and are nutritionally regulated at various steps. Germline stem cells and their early progeny proliferate to form a 16-cell cyst which is encapsulated by a monolayer of the somatic follicle cells to form an egg chamber that grows and develops into a mature oocyte. Using genetic mosaic analysis, we showed that mitochondrial fatty acid oxidation genes are not required in either the germline or follicle stem cell lineages. By contrast, glycolysis is required cell autonomously for germline stem cell maintenance, survival of 16-cell cysts, and growth and survival of egg chambers. Glycolysis is required in the early follicle cells for survival and more differentiated follicle cells for their proliferation and chromatin state. Interestingly, both stem cell lineages rely on each other›s metabolism. Glycolysis-defective follicle cells slow the growth of underlying wildtype germline cysts. Conversely, if glycolysis is disrupted in either the germline or follicle cells, the germline cysts are not properly encapsulated by follicle cells, potentially due to disrupted notch signaling between the two cell types. Surprisingly, glycolysis is not required for germline stem cell, cystoblast, or cyst proliferation, nor for proliferation of early dividing follicle cells. In the few surviving 16-cell germline cysts without glycolysis, entry into meiosis, oocyte specification, and histone modifications (specifically H3K9ac and H3K4me3) are unaffected. These results imply that other metabolites may be required for these specific processes, or that cells at these stages are metabolically flexible. We are currently investigating these possibilities, as well as the mechanisms of the specific metabolic requirements, and how they change under different physiological conditions, such as nutrient scarcity and exercise.

81 **Diacylglycerol Metabolism Dictates Enteric Pathogen Clearance** Xiaotong Li[1,2], Jason Karpac[1,2] [1]Department of Biology, Texas A&M University, [2]Department of Cell Biology and Genetics, Texas A&M University

Lipid metabolism is crucial for cellular function, playing a vital role in energy storage, membrane composition, and cellular signaling. Lipids are also increasingly recognized as important modulators of immune responses to bacterial infection through a variety of mechanisms, influencing host defenses to shape host-pathogen susceptibility. Here, we highlight a precise mechanism by which specific lipid metabolic pathways dictate pathogen clearance to alter host survival after infection. We demonstrate that enteric infection with *Pseudomonas entomophila* (*Pe*) induces significant neutral lipid accumulation in the *Drosophila* midgut, with lipidomic analysis identifying 1,2-diacylglycerols (DAGs) as the primary lipid species elevated during infection. Transcriptomic profiling further reveals that enteric *Pe* infection upregulates the expression of genes involved in lipid anabolism within the midgut. Attenuation of lipogenic genes, particularly those responsible for DAG synthesis or genes related to lipid transport within midgut enterocytes (ECs), inhibits lipid accumulation and decreases host survival after infection. Additionally, dietary supplementation with oleic acid enhances lipid accumulation and promotes survival following *Pe* infection, while inhibiting lipolysis in ECs results in increased lipid levels and enhanced survival. We found that these lipid-dependent processes shape host-pathogen susceptibility through influencing defecation rates after infection, thus dictating enteric pathogen clearance from the midgut. 1,2-DAGs are important signaling lipids, previously shown to directly regulate protein kinase C (PKC) function. We also found that lipid transport from ECs to midgut visceral muscle (VM) is required to regulate PKC levels and calcium flux, thus impacting muscle contraction in the VM following *Pe* infection. This lipid-and PKC-mediated VM contraction ultimately dictates enteric pathogen clearance through regulation of defecation. Overall, we uncover a novel mechanism whereby specific lipid metabolic pathways, likely shaping DAG levels and DAG-dependent signaling, influence pathogen clearance to direct host-pathogen susceptibility.

82 **Repetitive genomic elements shape adaptive evolution in *Drosophila melanogaster*** Alejandra Samano, Mahul Chakraborty Texas A&M University

Understanding the molecular and evolutionary mechanisms driving adaptation is a central challenge in evolutionary genetics. Genome structural variants (SVs), particularly repetitive elements like copy number variants (CNVs) and transposable elements (TEs), provide potential sources of adaptive variation. However, standard population genetic methods, which rely on short reads and a single linear reference genome, often obscure the role of these repetitive SVs in adaptation. Nearly 30% of the *Drosophila melanogaster* genome is repetitive, and this species has adapted to diverse environments, from its ancestral range in tropical Sub-Saharan Africa to temperate, cosmopolitan regions. Flies from temperate, higher-latitude populations exhibit trait differences such as variation in body and wing size, thermal stress tolerance, lifespan, diapause incidence, and fecundity compared to their tropical counterparts. These clinal patterns are observed across multiple continents, suggesting that these traits have evolved in response to new environmental selection pressures. Despite the observed adaptive phenotypic variation in *D. melanogaster*, the underlying genetic basis remains largely unknown. The genes involved in insulin signaling, such as the *Insulin-like receptor* (*InR*), are known to regulate life history traits, and TEs and CNVs could contribute to phenotypic differences between tropical and temperate populations. However, the lack of an SV map for African populations has hindered the investigation into this hypothesis. To address this gap, we created a comprehensive map of genetic variation by integrating newly assembled African reference genomes, built using using long-read sequencing, and existing cosmopolitan genomes, allowing us to explore the role of candidate genes like *InR* in adaptive phenotypic variation. We identified 13 TE insertions affecting cis-regulatory sequences in *InR*, some of which are private to populations and may explain functional differences between populations. We show that one TE, a Foldback (FB) element, inserted in a female-specific enhancer that drives *InR* expression in the leg muscles. This TE is present at high frequency in cosmopolitan populations but is low frequency or absent in ancestral African populations. Replacing the FB+ insertion allele with an FB- allele in the same genetic background using CRISPR-Cas9 reduced *InR* expression in female leg muscles but did not affect its expression in males. Our findings underscore the role of multiallelism and hidden SVs in driving functional variation underlying adaptive traits, including a trait that exhibits sexually dimorphic expression.

83 **ProteoCast: Proteome-wide prediction of the Functional Impact of Missense Variants in Drosophila melanogaster** Marina Abakarova[1,2], Arnaud Lierhmann[1], Maria Ines Freiberger[1], Michael Rera[3], Elodie Laine[1,4] [1]Sorbonne University, [2]Inserm, [3]CNRS, [4]Institut universitaire de France

Dissecting the functional impact of genetic mutations is essential to advancing our understanding of genotype-phenotype relationships and identifying new therapeutic targets. Despite the progress in sequencing and CRISPR technologies, proteome-wide mutation effect prediction remains challenging. To address this, we introduce **ProteoCast**, a computational method for proteome-wide classification of genetic variants and functional protein site identification. It relies solely on evolutionary information, leveraging protein sequence data across organisms. We used it to predict variant effects on the entire *Drosophila melanogaster* proteome.

Using ProteoCast, we generated mutational landscapes for 22,169 Drosophila proteoforms, categorizing over 293 million amino acid substitutions as functionally neutral, uncertain, or impactful. The full dataset is accessible on Zenodo (https://doi.org/10.5281/zenodo.10995110). We validated our predictions with observed natural polymorphisms in the Drosophila Genetic Reference Panel (DGRP) and Drosophila Evolution over Space and Time (DEST) datasets, along with FlyBase's developmentally lethal mutations. Notably, 86% of known lethal mutations were classified as impactful or uncertain, whereas only 13% and 18% were observed in DGRP and DEST, respectively.

ProteoCast provides confidence metrics at both protein and residue levels to help users make informed decisions. It enables identifying functional sites even in low-resolution (pLDDT < 70) AlphaFold2 structural regions. We further analyze sensitivity at residue and segment levels in low-pLDDT regions. Namely, it detects evolutionary pressure in about one third of 40.5K annotated post-translational modifications and 83% of the ~90 known short linear motifs. A case study of the Delta protein within the Notch signaling pathway confirms this, as ProteoCast accurately identifies essential residues and regulatory motifs within intrinsically disordered regions (IDRs), demonstrating its value for functional genomics.

Beyond providing predictions, we analyze input alignment information content, revealing that over 70% of potential substitutions appear in MSAs for more than half of the proteoforms, with nearly complete mutational landscapes explored for ~1000 proteoforms (591 genes). Proteins with fully covered landscapes tend to be more enriched in essential functions, more prone to lethal mutations, and exhibit limited natural polymorphism in DGRP lines.

ProteoCast is a scalable and efficient approach for proteome-wide mutation effect prediction. A public dataset and workflow will soon be available, fostering a broad exploration of gene function, mutation impacts, and translational applications across diverse species.

84 **Reorganization of the apical extracellular matrix underlies morphological diversification in Drosophila genital structures** Ben J Vincent[1], Lance Davidson[2], Mark Rebeiz[3] [1]Biological Sciences, California State University – Los Angeles, [2]Bioengineering, University of Pittsburgh, [3]Biological Sciences, 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 how body parts evolve. This genital structure exhibits staggering diversity among the *Drosophila melanogaster* subgroup, including the sister species *Drosophila mauritiana* and *Drosophila simulans*. We can also track posterior lobe development by dissecting and staining pupal terminalia in mutliple species. Previous work has shown that the posterior lobe develops in *Drosophila melanogaster* as a result of cell elongation, 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*. Using immunohistochemistry and confocal imaging, we quantified differences in posterior lobe morphology between these species and determined when these differences arise during pupal development. By labeling the aECM with fluorescent lectins, we found that it associates with the presumptive posterior lobe and during early development, and these attachments are more extensive in *Drosophila simulans*, the species with the larger lobe. The aECM also associates with a neighboring genital structure, the clasper, that also exhibits morphological differences between species. Using hybridization chain reaction to measure the *dumpy* gene expression pattern at cellular resolution, we found that this pattern is expanded in posterior lobe cells in *Drosophila simulans* compared to *Drosophila mauritiana*. These results suggest that morphological differences between species are at least partially controlled at the level of transcriptional regulation. Finally, we measured the gene expression patterns for other aECM components using *in situ* hybridization in both species. We find that some of these genes serve as markers for particular genital structures, and others may exhibit regulatory divergence between species. Our results suggest that alterations in extracellular matrix organization may underlie morphological differences between species, and that heterogeneity within the matrix may have functional consequences for genital development and evolution.

85 **A novel gene encodes a secreted protein that specifies variation in a rapidly evolving male reproductive structure** Md Golam Azom[1], Thomas Buckman[1], Erica Nadolski[2], Angelica Harper[2], John Masly[2] [1]University of Oklahoma, [2]School of Biological Sciences, University of Oklahoma

In the modern era of molecular biology, the study of development through the lens of evolution is a powerful way to dissect molecular mechanisms underlying the diversity of life. *Drosophila* male terminalia provides a unique and rich model system to study phenotypic divergence of very closely related species. Among the four sister species of the *Drosophila melanogaster* species complex— *D. melanogaster (mel), D. simulans (sim), D. sechellia (sec), and D. mauritiana (mau)*— males have evolved novel reproductive structures called the epandrial posterior lobes (ePLs). The bilaterally symmetrical ePLs are cuticular extensions from both sides of the male genitalia that insert between the seventh and eighth abdominal segments of females during mating to ensure a firm genital coupling during copulation. Although a few genes that specify species divergence in ePL morphology have been identified, less is known about the mechanisms by which these genes direct variation in ePL development. Using an interspecific genetic mapping approach, we identified a novel gene, *Goldilocks (Glds)*, that negatively regulates ePL size. The sequence features of *Glds* include a 51 base pair region that encodes a Signal Peptide (SP) at the 5' end of the Glds protein. We tested the function of this region in directing secretion of the Glds protein and found that Glds is secreted in all four species and that the SP sequence is both necessary and sufficient for secretion. Interestingly, the SP sequences in *sim* and *sec* share one unique amino acid (AA) substitution and *mau* has a different unique AA substitution compared to *mel*. A secretion assay for Glds showed that the AA substitution in *mau* directs significantly greater Glds secretion rate compared to the other species. Our results also suggest that additional species-specific AA substitutions in Glds outside of the SP sequence might contribute to secretion rates. CRISPR-Cas9 genome editing experiments are underway to test the phenotypic effects of species-specific substitutions in *Glds* along with cross-linking mass spectrometry experiments to identify candidates for Glds protein interactors.

86 **Evolutionary history of two X chromosome meiotic drivers in *Drosophila affinis*** Anjali Gupta, Robert L Unckless University of Kansas

Meiotic drivers are selfish genetic elements that bias gametogenesis to enhance their own transmission, cheating Mendelian segregation. When located on a sex chromosome, such drivers can skew progeny sex ratios, creating a female-biased population and diminishing average population fitness. In *Drosophila affinis*, two distinct X-linked meiotic drivers, with characteristic karyotypes distinguishable using unique inversions, segregate in wild populations (~10% frequency). The X chromosome in *D. affinis* is a neo-sex chromosome formed by the fusion of the ancestral X with a former autosome resulting in 40% of the genome being X-linked. We hypothesize that these inversions prevent recombination across the entire X chromosome and create distinct X chromosome haplotypes in *D. affinis*. Using whole genome sequencing of wild-caught male flies (with and without meiotic drive), we analyzed (i) the origin of the meiotic driving and non-driving X chromosomes, (ii) the divergence between the meiotic driving and non-driving X chromosomes, and (iii) whether driving X chromosomes accumulate greater genetic load, to assess the impact of meiotic drive on the genome evolution in *D. affinis*.

87 **Chromosome pairing in *Drosophila* hybrids** James G Baldwin-Brown, Nitin Phadnis Biology, University of Utah

The question of how homologous chromosomes find each other and pair up is a profoundly basic yet unsolved question. Although homologous chromosomes pair only during meiosis in most eukaryotes, homologous chromosomes are paired in all cells in Dipteran insects, included in somatic cells. Polytene chromosome squashes from interspecies hybrids between *D. melanogaster* and *D. simulans* show predictable regions of asynapsis across the genome. Here, we used Hi-C to measure chromosome pairing across the genome in hybrids and pure species. We find that the loss of homologous chromosome pairing is specific to polyploid cells, and that pairing is reduced across the entire genome in hybrids. Our results suggest that heterozygosity for species origin of chromosomes is sufficient to drive asynapsis in an otherwise well-paired genome. Further, using super-resolution microscopy and allele-specific oligo paint probes, we find that homologous chromosomes get de-mixed but that sister chromatids of each homologous chromosome remain well-mixed, thus producing the pattern of asynapsis on hybrids. Our results have important implications for understanding the mechanisms of homologous chromosome pairing, including during meiosis.

88 **Selective response of mitochondrial and nuclear genomes to an OXPHOS inhibitor in experimental populations of *Drosophila*** Leah J Darwin[1], Yevgeniy Reynes[2], Rebecca Bachtel[2], Faye Lemieux[2], David Rand[2] [1]Center for Computational Biology, Brown University, [2]Ecology Evolution and Organismal Biology, Brown University

When a population is introduced to a new selective environment, understanding the mechanisms underlying the adaptive response can be challenging, especially when it entails subtle changes in allele frequencies across many genes. The extent to which such a "polygenic" adaptive response to a particular environment may be repeatable –and thus predictable– depends on the genetic composition of the founding population and the number of genetic targets available to achieve a specific adaptive phenotype. Here, we use replicate experimental populations of *Drosophila melanogaster* to study the repeatability of polygenic adaptation across the nuclear (nDNA) and mitochondrial (mtDNA) genomes. To target the two sets of genes, we used rotenone, an insecticide that inhibits the oxidative phosphorylation system (OXPHOS), which relies on both the mitochondrial and nuclear genomes. We constructed 24 outcrossed replicate populations comprising of mtDNA variation from 24 different strains of *D. melanogaster*, 1 strain of *D. simulans*, and 1 strain of *D. yakuba,* as well as nDNA variation from two common North American *D. melanogaster* lines *OregonR* and *DGRP375* and from natural Zimbabwe and Beijing populations. After 40 generations of exposure to a rotenone diet, development time assays indicated that all 12 experimental populations had developed resistance to rotenone. We used pooled sequencing at six different time points across all 24 experimental and control populations to quantify allele frequencies over time. We observed subtle, parallel, changes in mitochondrial haplotypes across experimental populations that were not observed in control populations. Changes in mitochondrial haplotype frequencies suggest that there is selection against more divergent haplotypes (*D.yakuba* and *D.simulans*). We also identified several nuclear variants as potentially adaptive to rotenone, suggesting a polygenic adaptive response in the nuclear genome as well. Of particular interest, we identified a parallel change in the gene Rab11, a strong candidate for driving adaptation to rotenone, in light of earlier studies showing that rotenone exposure could induce sporadic Parkinson's disease (PD) in *Drosophila*. Rab11 is notable as it interacts with the PD-associated gene Parkin.

89 **Evolved suppression of recently expanded retroelements** Deep Sangha, Kevin Wei Zoology, University of British Columbia

Transposable elements (TEs) occupy large swaths of eukaryotic genomes and are some of the most rapidly changing constituents. Repression of TEs requires the production of complementary piRNAs for post-transcriptional degradation and the local formation of heterochromatin for transcriptional silencing. In Drosophila, H3K9me3, one of the key repressive histone modifications, is established during zygotic genome activation. Previously, ChIP-seq on Drosophila miranda embryos revealed that while the bulk of H3K9me3 appear at embryonic stage 4 (nuclear cycle 10-13), a small number of retroelement families show position-specific nucleation of H3K9me3 at stage 3, the onset of ZGA, which then spread to neighboring nucleosomes. Because TE families with such targeted H3K9me3 nucleations have lower expression but, counterintuitively, higher copy number, we hypothesize that they are recently active elements that are now being targeted for silencing. Consistently, phylogenetic comparisons to the sister species D. pseudoobscura revealed that the elevated copy number resulted from recent expansions specific to D. miranda. Further, in D. pseudoobscura, the same families, despite fewer copies, are more highly expressed and, curiously, do not nucleate H3K9me3 during early embryogenesis, suggesting that early nucleation may have arisen in D. miranda as an adaptation to silence these expanding TEs. While piRNAs have been proposed to mediate heterochromatin formation at TE insertions, we found no association between the abundance of piRNA mapping and the nucleation sites suggesting a piRNA independent mechanism underlying targeted H3K9me3 nucleation. Instead, we propose that maternally deposited DNA-binding proteins target specific motifs in these TEs to recruit histone methyltransferases inducing restricted H3K9 nucleation. Consistent with this possibility, we observed targeted nucleation only when D. miranda is the mother of hybrid embryos produced by reciprocal crosses of the two species. Overall, our work reveals the potential for countermeasures beyond the piRNA pathway to emerge amidst insidious and selfish spread of TEs.

90 **Single-Domain Antibodies for Reducing Pathological α-Synuclein and Mitigating Disease Deficits** Pragati Sharma[1], Hyung Don Ryoo[2] [1]Department of Neuroscience and Physiology, and the Neuroscience Institute, New York University Grossman School of Medicine, [2]Department of Cell Biology, New York University Grossman School of Medicine

Synucleinopathies include Parkinson's Disease, Lewy Body Dementia, and Multiple System Atrophy. These disorders are primarily characterized by motor and neuropsychiatric symptoms, imposing a significant socioeconomic burden. Currently, treatment options for synucleinopathies are limited to symptomatic relief, with no available cures. Given the association of familial synucleinopathies with α-synuclein (α-syn) gene mutations and replication, therapeutic strategies aimed at reducing α-syn accumulation are of high interest. Immunotherapies targeting α-syn has been found to be effective in ameliorating disease pathology in preclinical models with several being examined in clinical trials. However, clinical IgG antibodies currently face challenges in crossing the blood-brain barrier (BBB) due to their large size (~150 kDa). Smaller antibody fragments, such as single-chain variable fragments (scFvs, ~25 kDa) or single-domain antibodies (sdAbs, VHH ~15 kDa), offer improved brain penetration, albeit with a shorter half-life. Promising in vitro studies on α-syn-targeting sdAbs have shown potential, though in vivo evidence remains limited. Recently, we have developed sdAbs against human α-syn that have great potential for in vivo diagnosis and are effective in degrading toxic α-syn in culture and in vivo (Jiang Y et al., Sci Adv, 2023; Jiang Y et al., Mol Neurodegener, 2024). Interestingly, these sdAbs target the NAC domain of α-syn, whereas the whole IgGs in clinical trials target the N- or C-terminus of α-syn. To further assess their in vivo therapeutic potential, we generated five different anti-α-syn sdAb transgenic flies and a control sdAb that does not bind to α-syn (targets Dengue virus). We then crossed these flies with a fly model expressing wild-type human α-syn (Rousseaux MWC et al., J Neurosci, 2018). We found that certain anti-α-syn sdAbs reduced the levels of phosphorylated serine 129 of α-syn, which is a pathological α-syn epitope, and prevented α-syn induced loss of dopaminergic neurons. These therapeutic benefits correlated with their ability to suppress locomotor defects. In conclusion, this study advances novel immunotherapies for synucleinopathies by demonstrating the potential of sdAbs to effectively target and reduce pathological α-syn in vivo, resulting in neuronal and functional improvement.

91 **Pathogenic CryAB Mutations in Muscle Suggest a Novel Mechanism for Amyloid Spread via Extracellular Vesicles** Erika Geisbrecht, Ziwei Zhao Biochemistry & Molecular Biophysics, Kansas State University

Amyloid fibrils are insoluble protein aggregates commonly linked to neurodegenerative diseases, including Alzheimer's and Parkinson's disease. While amyloid structures have been extensively observed in the brain, they are also found in other organs such as the heart, kidney, and liver, a condition known as amyloidosis. Amyloid deposits in skeletal muscle, however, remain less documented. Human mutations in the small heat shock protein αB-crystallin (CryAB) result in cataracts, cardiomyopathies, and myofibrillar myopathies, all characterized by the presence of protein aggregates. In this study, we expressed four human pathogenic alleles of CryAB in *Drosophila* muscle tissue to define how altered chaperone activity impacts protein quality control. Each of these CryAB mutations impaired autophagic flux, resulting in protein aggregates marked by ubiquitin (Ubi) and p62/Sequestosome-1 (SQSTM1). In addition, mutations located in the conserved CryAB α-crystallin domain (ACD) or a hyperphosphorylated form of CryAB, led to the formation of unique, CryAB-positive inclusions that colocalized with an amyloidogenic form of the human intermediate filament protein desmin. Transmission electron microscopy (TEM) and Congo red staining confirmed that these disease-causing CryAB mutations formed amyloid-like fibrillar structures. Unexpectedly, these CryAB amyloid inclusions also stained positive for multivesicular body (MVB) and extracellular vesicle (EV) markers. The findings together expand our understanding of amyloid fibril architecture in vivo and provide a possible pathogenic mechanism for the spread of protein aggregates in disease progression, possibly dependent on phosphorylation.

92 **Breakdown of Neuronal Glycogen Protects Against Tauopathy by Shunting Sugar to the Pentose Phosphate Pathway** Sudipta Bar, Pankaj Kapahi, Kenny Wilson Buck Institute for Research on Aging

The abnormal accumulation of microtubule-associated protein tau (MAPT) is well-documented in neurodegenerative diseases such as Alzheimer's disease, frontotemporal dementia, and other conditions collectively known as tauopathies. In this study, we demonstrate that dietary restriction (DR), a potent intervention known for extending lifespan and providing neuroprotection, significantly reduces pathology in a Drosophila melanogaster model of tauopathy, where a mutant form of human tau is overexpressed. Our findings indicate that DR promotes neuroprotection by enhancing glycogen breakdown in neuron. We observed impaired glycogen metabolism in both fly and human models of tauopathy, and we found that activating glycogen phosphorylase (GlyP) to increase neuronal glycogen breakdown reverses tauopathy phenotypes. Interestingly, increasing glycogen catabolism in neurons redirects glucose flux towards the pentose phosphate pathway instead of glycolysis, thereby mitigating oxidative stress. We further showed that DR activates GlyP through the cAMP-mediated protein kinase A (PKA) pathway, and pharmacological activation of this pathway alleviates tau pathology. These results suggest a potential therapeutic strategy for managing tauopathies through targeted modulation of glycogen metabolism.

93 ***Drosophila* modeling of insomnia- and cardiovascular disease-associated genes finds excessive sleep correlates with aberrant cardiac function** Torrey Mandigo[1,2], Farah Abou Daya[1], Suraj Math[1], Lily Ober[3], Dev Patel[3], Girish Melkani[3], Richa Saxena[1,2], James Walker[1,2] [1]Center for Genomic Medicine, Massachusetts General Hospital/Harvard University, [2]Broad Institute, [3]University of Alabama at Birmingham

Insomnia disorder occurs in 10-20% of the population and is an important risk factor for incident cardiovascular disease (CVD) conferring a >2-fold increased causal risk. However, the underlying pathways and mechanisms linking the two remain poorly understood. We utilized the recent advances in large-scale sleep GWAS to identify human genes on haplotypes associated with insomnia and other sleep traits. From these candidate loci, we prioritized those with only one or two genes located within the haplotype and/or, with prior evidence of links to CVD, coronary artery disease (CAD), or cardiometabolic disease (CMD). After identifying *Drosophila* orthologs of these candidate genes, we used RNAi to knock down expression of each ortholog in neurons or the heart to assess their cell-autonomous effects on sleep and cardiac function respectively. Since mendelian randomization studies in humans have found insomnia symptoms causally contribute to CAD, we also looked at cell non-autonomous effects of neuronal KD on cardiac function and heart KD on sleep. Our cell non-autonomous experiments uncovered a strong association between excessive sleep and aberrant cardiac function. These cell non-autonomous experiments highlight various pathways that are essential for the functions of both tissues as well as potential pathways conferring cell non-autonomous regulation of sleep and cardiac function, providing a shortlist of therapeutically relevant genes and pathways that link insomnia, sleep and CVD.

94 **Early life social isolation primes flies for more rapid TDP-43 dependent neurodegeneration later in life** Joshua Dubnau[1], Swetha Murthygowda[2], Kyle Huyghyue[3] [1]Department of Neurobiology and Behaviour, Department of Anaesthesiology, Stony Brook University, [2]Department of Anaesthesiology, Stony Brook University, [3]Undergraduate Biology, Stony Brook University

Amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) are fatal neurodegenerative disorders that include progressive motor defects and cognitive dysfunction. One of the key pathophysiological hallmarks of the disease is loss of nuclear localization and abnormal cytoplasmic aggregation of TAR DNA-binding protein 43 (TDP-43). In human subjects and animal models, it is thought that pathological aggregates of TDP-43 can trigger spread of toxicity to nearby neurons or glial cells, resulting in a propagation of the disease through neural tissue. TDP-43 pathology is observed in affected brain regions of ~95% of ALS and ~40% of FTLD patients. While some cases are inherited, most cases are sporadic, with no known genetic causes, suggesting important contributions from environmental factors. Epidemiological evidence indicates that PTSD, anxiety, depression and loneliness are associated with increased risks of neurodegenerative disease, although causality cannot be not established in humans. We examined the impacts of chronic social isolation, a paradigm previously shown to cause sleep loss in flies. We found that early life social isolation is sufficient to sensitize animals to the effects of TDP-43 pathology in glial cells. By contrast, experiencing the same social isolation paradigm in more mature animals has no effects on the rate or severity of neurodegeneration. When applied during the first few days after eclosion, social isolation exacerbates the effects of subsequent induction of pathological levels of TDP-43 in sub-perineural cells (SPG). Using confocal imaging, we determined that early life isolation increases the rate of propagation of aggregated TDP-43 protein from SPG to nearby neurons, and further shortens life span compared to animals of the same TDP-43 expressing genotypes that had never experienced isolation. Social isolation also increases the rate of replication of an engineered reporter of the mdg4 endogenous retrovirus. Such endogenous retrovirus expression plays a key role in triggering both aggregation and inter-cellular spread of TDP-43 pathology. Our findings demonstrate that social isolation during a critical period early in life is acts as a primer that accelerates the rate and severity of neurodegeneration in response to subsequently induced toxic levels of TDP-43. These findings suggest the possibility of a causal link between psychological stressors such as loneliness and risk of neurodegenerative diseases in humans.

95 **Investigating the role of mitochondrial LIPT1 and GLUD2 disease-associated variants in flies** Bhagyashree Kaduskar[1,2], Christina Miyake[3], Mary Koenig[4], Hugo Bellen[1,2,5,6] [1]Department of Molecular and Human Genetics, BAYLOR COLLEGE OF MEDICINE, [2]Jan and Dan Duncan Neurological Research Institute, Texas Children's Hospital, Houston, [3]Department of Pediatrics (Cardiology), BAYLOR COLLEGE OF MEDICINE, [4]Center for the Treatment of Pediatric Neurodegenerative Disease, UTHealth, [5]Department of Neuroscience, Baylor College of Medicine, [6]Program in Development, Disease Models and Therapeutics, Baylor College of Medicine

Mitochondrial disorders due to a dysfunctional TCA cycle are linked to many human diseases, and flies are an important model to study the mechanisms. Here, we focus on two mitochondrial proteins, LIPT1 and GLUD2, which are crucial for TCA cycle function. LIPT1 is necessary for the lipoylation of enzymes including pyruvate dehydrogenase (PDH) and OGDH (α-ketoglutarate dehydrogenase). Deficiency in *LIPT1* leads to a rare disorder that presents with reduced PDH and OGDH activity, increased lactate and α-ketoglutarate (α-KG), and a progressive neurodegeneration, and early death. We currently have two patients with compound heterozygous mutations in *LIPT1* who present with acute lactic acidosis, α-KG accumulation, and neuronal phenotypes. *GLUD2* encodes a glutamate dehydrogenase 2, converting glutamate to α-KG and ammonia. We have identified 5 patients with potential pathogenic mutations in *GLUD2* and they present with syncope, easy bruising, arrythmia, and in some cases sudden death.

In the past, our lab identified different phenotypes for the loss of genes that lead to decreased α-ketoglutarate (α-KG) or a buildup of α-KG in flies. Reduced levels of α-KG leads to a decreased function of Synaptotagmin1 and decreased synaptic transmission which can be partially rescued by dietary α-KG supplementation. Increased levels of α-KG due to loss of OGDH function, on the other hand, lead to mTOR pathway activation which can be partially reversed with Rapamycin.

Flies have single ortholog of *LIPT1* (*Lipt1*) and *GLUD2* (*Gdh*). Loss of *Lipt1* or *Gdh* is embryonic lethal. Additionally, a neuronal knockdown of *Lipt1* leads to a severe neurodegenerative phenotype, locomotion defects and a shorter lifespan which is not dissimilar to symptoms observed in patients with *LIPT1* deficiency. Using the CRiMiC T2A-Gal4 strategy, we also show that *Lipt1* is expressed in neurons and some glia whereas *Gdh* is expressed primarily in a subset of neurons. These CRiMiC T2A-Gal4 alleles are loss of function and cause embryonic lethality. In the background of these T2A-GAL4 alleles, we are currently expressing the human *LIPT1* or *GLUD2* reference as well as potential disease variants to assess the strength of rescue as well as nature of the potential disease-causing variants identified in the patients. Moreover, using these human *LIPT1* and *GLUD2* reference or variants expressing flies, we will be testing various drugs (Rapamycin, NACA, Triheptanoin, *etc.*) as well as dietary interventions that may ameliorate the effects of α-KG dysregulation. In parallel, we are assessing the metabolic profile of the *LIPT1* or *GLUD2* variants in human iPSC derived cells and testing the effect of drugs on these profiles.

96 **Drosophila Model of HPV18-Induced Pathogenesis Reveals a Role for E6 Oncogene in Regulation of NF-κB and Wnt to Inhibit Apoptosis** RAMI N. HASSAN[1], Mehrnaz Afkhami[1,2], John P. Masly[1], Harrison Brown[3], Quincy P. Collins[4], Michael J. Grunsted[5], Mojgan Padash Barmchi[1] [1]School of Biological Sciences, University of Oklahoma, [2]Department of Molecular Biology and Genetics, Cornell University, [3]Children's Medical Center Research Institute, UT Southwestern Medical Center, [4]Department of Cellular and Physiological Sciences, Faculty of Medicine, The University of British Columbia, [5]College of Medicine, University of Oklahoma Health Sciences Center

Cancers caused by high-risk human papillomavirus (HR-HPVs) remain a significant health threat resulting in more than 300,000 deaths, annually. Persistent expression and action of two HPV oncogenes, E6 and E7, are necessary for cancer development and progression. The main function of E6 is cell immortalization through the inhibition of cell death pathways. However, the molecular mechanism is not fully understood. Here, using a *Drosophila model* of HPV18E6 and the human UBE3A-induced pathogenesis, we show that the anti-apoptotic function of E6 is conserved in *Drosophila*. We demonstrate that the *Drosophila* homologs of human NF-κB transcription factors, Dorsal and Dif are proapoptotic. They induce the expression of Wingless (Wg, the *Drosophila* homolog of human Wnt), leading to apoptosis. Our results indicate that the E6 oncogene inhibits apoptosis by downregulating the expression of Wg, Dorsal, and Dif. Additionally, we find that Dorsal and Dif, not only promote apoptosis but also regulate autophagy and necrosis. Dorsal promotes autophagy while Dif counteracts it, inducing the formation of acidic vacuoles and necrosis. Interestingly, although E6 blocks the proapoptotic function of Dorsal and Dif, it lacks the ability to inhibit Dif-induced necrotic cell death. Given the high conservation of NF-κB transcription factors, our results provide new insight into potential mechanisms mediated by NF-κB to intervene with cell immortalization action of E6 oncoprotein in HPV-infected cells.

97 **Translation regulation by ribosome recycling factors in retinal development and disease** Katherine E Querry[1], Narayanan Nampoothiri[1], Christopher Garbark[2], Nina Gralewski-Goel[2], Abigail Carney[2], Mykola Roiuk[3], Aurelio Teleman[3], Deepika Vasudevan[2] [1]Cell Biology, University of Pittsburgh School of Medicine, [2]University of Pittsburgh School of Medicine, [3]German Cancer Research Center

Photoreceptors of the visual system rely heavily on endoplasmic reticulum (ER) stress response pathways for their function due to their high secretory burden. Consequently, disruption of these pathways results in retinal degeneration. We had previously demonstrated that loss of the ER stress response transcription factor, ATF4, results in age-dependent retinal degeneration. ATF4 expression is largely regulated by the 5' leader region of the encoding mRNA but the extra-ribosomal factors that enforce such regulation are not well studied. To discover such factors, we performed a *Drosophila* RNAi screen which identified the ribosome recycling factor, Hbs1, as a potential regulator of ATF4. Mutants for Hbs1 and its dimerization partner, Pelota, resulted in reduced ATF4 activity. Interestingly, human patients with HBS1 deficiency have been reported to display retinal degeneration amongst other neurodevelopmental defects. Consistently, electroretinogram (ERG) analysis of *Hbs1* mutant *Drosophila* shows age-dependent retinal degeneration and indicates defects in either lamina neuron development or photoreceptor-lamina synapse formation. RNAi depletion of *Hbs1* in lamina neurons was sufficient to phenocopy ERG defects seen in *Hbs1* mutants; we are currently testing whether ATF4 expression in these cells is sufficient to rescue these defects.

The ATF4 mRNA 5' leader contains two upstream open reading frames (uORFs) that regulate translation of the ATF4-encoding main ORF. Translation is typically initiated at uORF1 and reinitiated either at uORF2 under homeostasis or at the ATF4 ORF when ER stress response is activated. Thus, translation initiation at the ATF4 ORF is dependent on successful translation termination at uORF1. Since Hbs1 and Pelota form a heterodimeric complex to recycle post-termination ribosomes, we hypothesize that these factors regulate reinitiation at the ATF4 ORF by mediating efficient termination at uORF1. Consistent with this hypothesis, siRNA knockdown of Hbs1 or Pelota in human cells showed decreased expression of an ATF4 5' leader reporter. Mutating the uORF1 stop codon abolished the Hbs1-Pelota dependence of the reporter, further supporting our hypothesis. Altogether, our data present a new model for understanding retinal degeneration in Hbs1 deficient patients and posit that such degeneration may result from Hbs1-mediated translation regulation of ATF4.

98 **Regulation of Drosophila Muscle Stem Cells by The Octopaminergic Nervous System** Ammar Aly[1], Pejmun Haghighi[2] [1]The Buck Institute for Research on Aging, [2]Haghighi Lab, The Buck Institute for Research on Aging

Stem cells must balance their dual functions of differentiation and self-renewal in order to achieve tissue homeostasis, a process that is essential for the maintenance of all multicellular life. Disruptions to this delicate balance are increasingly thought to underlie the pathogenesis of numerous diseases, particularly those associated with aging. Although recent evidence suggests that the autonomic nervous system (ANS) may be a critical regulator of stem cell fate, the specific mechanisms by which the ANS influences stem cell biology—especially in muscle stem cells (MuSCs)—remain largely unexplored. To investigate the role of the ANS in stem cell regulation, we employed Drosophila adult muscle stem cells (MuSCs) and octopaminergic innervation, invertebrate equivalent of sympathetic adrenergic innervation, to create a robust model for investigating ANS-mediated regulation of stem cell function. Using our newly developed high-resolution, multispectral imaging protocols, we have conducted comprehensive mapping of autonomic innervation patterns across the entire adult fly body, revealing significant and progressive changes in innervation with age. This imaging approach allowed us, for the first time, to visualize direct synaptic contacts between ANS neurons and muscle stem cells, providing a previously unseen perspective on stem cell innervation. Our results demonstrate that octopaminergic neurons directly innervate MuSCs, and that this signaling is essential for promoting stem cell self-renewal and regenerative function. Further, we show that with age, the integrity of these connections diminishes, which correlates with an increase in stem cell quiescence and an impaired regenerative capacity. These findings highlight the importance of ANS input in maintaining stem cell homeostasis and suggest that loss of ANS support contributes to the age-associated decline in tissue repair and regeneration. By uncovering the critical role of direct ANS-MuSC synaptic connections, our study identifies novel regulatory pathways that control stem cell fate and function. These pathways represent potential targets for therapeutic interventions aimed at restoring stem cell vitality and combating age-related diseases. Additionally, our work advances our understanding of the broader mechanisms by which the nervous system influences stem cell biology across the lifespan.

99 **Evolution of gut symbionts over a single lifespan can reduce host longevity** Angela Xu, William Ludington CMDB, Johns Hopkins University

Microbiome-host imbalance, or dysbiosis, occurs as animals age, with studies suggesting that microbiomes from aged hosts might transfer frailty to naive young hosts. To test this hypothesis, we inoculated *Drosophila melanogaster* with a natural community of commensal Acetobacters and Lactobacilli isolated from a single wild-caught *D. melanogaster*. We measured fly lifespans, sampled their associated microbiota over time, and re-inoculated these sampled bacteria into young flies. Microbiota from old hosts caused 10% shorter lifespans, while microbiota from young flies produced normal lifespans. Because all strains persisted during the fly lifespan, we hypothesized that mutations arose in the bacteria during the fly's lifespan that accelerated aging. To test this, we sequenced the initial inoculants and the isolated strains from old flies. We found multiple independent mutations in a putative heptose glycosyltransferase gene in one of the Acetobacter strains across independent biological replicates of the aging experiment. These mutant strains were sufficient to reduce the host lifespan by 5 percent. By measuring the relative abundance of the mutant in co-colonization with the other species, we found that the mutant outcompetes other members as the host ages, triggering dysbiosis. RNAseq experiments indicate an early onset of reactive oxygen species stress in flies inoculated with the mutant bacteria. Our results demonstrate that a mutation arising in a single commensal bacterial species over the course of a host lifespan can negatively impact longevity. Therefore, a possible mechanism for the appearance of age-related dysbiosis and incipient host decline is through microbiome evolution.

100 **Microbiota-mediated suppression of a gut-derived decretin's expression promotes *Drosophila* larvae systemic growth upon malnutrition** Longwei Bai, Manon Picquenot, Jacques Montagne, Cathy Ramos, François Leulier Institut de Génomique Fonctionnelle de Lyon, Ecole Normale Supérieure de Lyon, CNRS UMR5242, Université Claude Bernard Lyon 1

Our previous findings unveiled that the association of undernourished germ-free (GF) *Drosophila* larvae with selected commensal bacteria, including *Lactiplantibacillus plantarum*^{WJL} (*Lp*^{WJL}), supports juvenile growth *via* promoting the systemic activities of *drosophila* Insulin-like peptides (dILPs). Nevertheless, how gut endocrine functions coordinate with systemic insulin signaling remains elusive. Here, we show that five enteroendocrine cells (EECs) in the anterior larval midgut respond to nutrient deprivation by up-regulating Limostatin (Lst), a decretin hormone previously characterized from corpora cardiaca in *Drosophila* adults. Upon malnutrition, gut-derived *lst* was dramatically induced in GF larvae, whereas its transcript levels were notably inhibited upon *Lp*^{WJL} association via the microbial amino acid provision and bacterial cell wall sensing by host cells. Consistently, we found that *lst*-deficiency or EEC-specific knockdown of *lst* significantly reduced the delay of larval systemic growth caused by malnutrition. More intriguingly, attenuated insulin signaling in EECs triggers the expression of *lst,* in line with the inhibition of larval systemic growth upon undernutrition. Taken together, our findings indicate that *Lp*^{WJL} suppresses the malnutrition-triggered upregulation of gut-derived *lst*, which in turn supports *Drosophila* larvae systemic growth through the regulation of insulin signaling by the gut-derived decretin Lst.

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101 **Diabetes and obesity regulated (DOR)/TP53INP1 regulates mortality, resilience, and ovarian senescence in *Drosophila melanogaster*** Tyler A.U. Hilsabeck, Dipti Verma, Sudipta Bar, Lindsay Gann, Kenneth A. Wilson, Pankaj Kapahi Buck Institute for research on aging

Late-life mortality refers to the mortality rates of older adults over time, providing insights into patterns of death in aging populations. Understanding the components and factors responsible for late-life mortality is imperative to inform interventions to improve health and extend lifespan. To identify the genetic regulators responsible for late-life mortality in *Drosophila*, an analysis of the lifespan periods of 160 fly strains in the DGRP was conducted. *GWAS* investigation led to the identification of Diabetes and Obesity-Regulated (DOR), which contains a locus associated with late-life mortality pathways. DOR's human orthologs, TP53INP2 and TP53INP1, are AD GWAS candidates with known or potential roles in multiple AD risk factors. Further, TP53INP1 has also been shown to regulate somatic granulosa cell (GC) cycle arrest in response to hypoxia. Since GCs play an important role in regulating the female reproductive system, it is hypothesized that DOR might play a substantial role in modulating female reproductive health. To further determine the role of DOR in the regulation of the aging of the organism and ovarian health, we inhibited DOR in the whole body of female *Drosophila* using DOR-RNAi and studied its impact on survival and late-life mortality which resulted in the shortening of the lifespan. Additionally, DOR downregulation resulted in compromised organismal health as evidenced by failure to climb properly and increased gut permeability associated with declining organismal health. Flies with DOR inhibition placed on starvation media were less able to survive under starvation conditions. To understand the mechanisms responsible for the decline of organismal health and early mortality due to DOR inhibition, RNA-seq was conducted from flies with compromised DOR. The most significantly upregulated and downregulated pathways included genes with enrichment for the *Toll* and *Imd* signaling, and ribosomal and oxidative phosphorylation pathways, respectively. Further, DOR inhibition led to increased expression of two senescence factor orthologs, *p53, dacapo (p21)*, as well as two senescence-associated secretory peptides, *upd1 (IL6) and mmp1.* To test whether the inhibition of DOR in a particular tissue would recapitulate the detrimental phenotypes observed, we abrogated DOR in multiple tissues and observed shortened lifespan repeatedly upon downregulating DOR in the ovaries. Additionally, ovary-specific inhibition of *DOR* led to a significant increase in the expression of senescent markers. Alternatively, overexpressing DOR showed lifespan extension, decreased gut permeability, and reduced senescence in the ovaries. Taken together, our studies suggest a significant role of DOR in the regulation of healthspan and lifespan metrics by modulating the reproductive function in *Drosophila*

102 **Developmental programming of respiratory complex levels determines lifespan** Beatriz Castejon Vega, Rachel Zussman, Ignacio Fernandez Guerrero, Alberto Sanz School of Molecular Biosciences, College of Medical, Veterinary and Life Sciences, University of Glasgow

Mitochondria are crucial for maintaining cellular homeostasis by producing energy. Beyond energy production, these organelles also function as vital communication hubs, synthesizing essential cellular components and orchestrating life-and-death decisions through apoptosis. Given their diverse roles, it is not surprising that mitochondrial deterioration is a fundamental hallmark of ageing, characterized by an accumulation of damaged mitochondria with diminished ATP production capacity and increased levels of Reactive Oxygen Species.

In this study, we demonstrate that development is the critical period during which mitochondrial activity determines the duration of adult life. Targeting Complex IV (CIV) subunits with RNA interference during the development of *Drosophila melanogaster* significantly shortens adult survival and reduces stress adaptation, whereas depletion limited to adulthood has minimal impact. This underscores the concept of a "developmental window of opportunity," a phase during which mitochondrial function must remain intact for enhanced longevity and stress adaptation.

To demonstrate the existence of this "developmental window of opportunity," we exclusively expressed an alternative oxidase (AOX) during development, which, although only partially complementing CIV activity, was sufficient to extend lifespan and reverse many transcriptomic and metabolomic changes induced by CIV dysfunction. Similarly, restoring CIV activity through the co-expression of a rescue construct carrying silent mutations during development successfully restored both CIV function and lifespan. Surprisingly, expression of the same rescue construct solely in adults failed to restore CIV activity. A thorough investigation using transcriptomics, proteomics, and data mining revealed that levels of respiratory complexes are established during fly development and remain constant throughout the adult lifespan.

Our findings suggest that short-lived organisms like *Drosophila melanogaster* have evolved strategies to adjust adult metabolism to the levels of mitochondrial respiratory complexes established during development, minimizing turnover during adulthood. We propose that this strategy maximizes reproductive output—a metabolism adapted to post-eclosion environmental conditions—at the expense of long-term survival, which is reduced by the accumulation of damaged mitochondria during ageing

103 **The *Drosophila melanogaster suppressor of black* mutation, *su(b)*, maps to Malonate Semialdehyde Dehydrogenase (MSDH), the final enzyme in the beta-alanine catabolic pathway** Eric P Spana, Isabela M. Aguilar, Kyra Y. Chen, Tomás S. Delgado, Erin Dollard, Madeleine B. Ganz, Makayla Gorski, Elizabeth Lukasz, Megan Maransky, Nhi N.Y. Nguyen, Layne O'Brien, Ethan B. Rehder, Yuzhe Yuan, Hishi Ulak Biology, Duke University

β-Alanine is a beta amino acid crucial for melanization and sclerotization in *Drosophila melanogaster* and during adult cuticle pigmentation is predominantly made from L-aspartic acid by the product of the *black* (*b*) gene. The *suppressor of black* mutation, *su(b)*, was first identified as "Black Suppressor" by Plough in 1928, lost, then re-isolated by Sherald in 1981. Mutations in *su(b)* suppress the hyper-melanization of the body and wings of *black* mutants in *D. melanogaster* in a recessive manner. *su(b)* mutants also have increased levels of β-alanine presumably due to reduced β-alanine catabolism. This allows β-alanine derived from a second pathway, the pyrimidine catabolism pathway, to be used for sclerotin production by *ebony*. We mapped the *su(b)* gene and identified the $su(b)^2$ allele as a premature stop codon in *Malonate Semialdehyde Dehydrogenase* (*MSDH*, CG17896), the final enzyme in β-alanine catabolism. Using a custom wing pigmentation quantification program, we demonstrated no statistically significant pigmentation difference between $su(b)^2$; b^1 and wild-type wings and found that $su(b)^2$ wings are slightly lighter than wild type. This is the first description of a catabolic pathway for melanin and sclerotin precursors being involved in Drosophila pigmentation.

104 **Single-cell CUT&Tag tracks cell type specific chromatin changes in the aging Drosophila intestine** Sarah M Leichter[1], Kami Ahmad[1], Steve Henikoff[1,2] [1]Division of Basic Sciences, Fred Hutchinson Cancer Center, [2]Howard Hughes Medical Institute

Aging in tissues is associated with the progressive decline of functionality and changes to the chromatin landscape. While most adult tissues comprise post-mitotic cells, the Drosophila intestine contains a population of somatic stem cells that repopulate the organ multiple times throughout the organism's lifespan and is a powerful system to study chromatin changes with aging due to its striking aging phenotypes: overproliferation of stem cells and break down of the barrier membrane. To probe the chromatin basis for progressive decline, we mapped the H3K27me3 histone modification via CUT&Tag in the Drosophila intestine at different ages and observed a drastic decrease in H3K27me3 over established Polycomb domains and promoters in aged tissue. We used single-cell combinatorial indexing CUT&Tag (sciCUT&Tag) to distinguish intestinal cell types and determine age-related changes in H3K27me3 for stem cells and for differentiated cells of the intestine. We found that enterocytes, the differentiated cells of the gut, show the greatest gains and losses of H3K27me3 in aged tissues, likely accounting for most of the changes in H3K27me3 in bulk CUT&Tag. In contrast, intestinal stem cells show few promoter and domain changes in H3K27me3, indicating that the stem cells of the intestine are resistant to age-related chromatin changes. Intriguingly, we find that the Unpaired domain– which encodes the ligands of the growth-stimulating JAK/STAT signaling pathway – is derepressed in aged intestinal stem cells, providing a potential mechanism for their increased proliferation in aged intestines. Additionally, we observe the formation of new H3K27me3 domains in aged enterocytes, one of which forms over Chitin synthase 2, an enzyme responsible for synthesizing chitin, a key component of the barrier membrane of the insect intestine. Our results suggest that critical repressive chromatin modifications underlie progressive decline with age in regenerative tissue.

105 **Aggregation of the Nuclear Envelope Protein Lamin in Aging and Disease** Cameron Call[1], Bismark Acquah[2], Alysia Vrailas-Mortimer[3,4] [1]Oregon State University, [2]Illinois State University, [3]Biochemistry and Biophysics, Oregon State University, [4]Linus Pauling Institute, Oregon State University

Aging is a natural process that most organism experience. One gene that plays a role in aging is lamin, which when mutated can give rise to a variety of age-dependent diseases, including the accelerated aging disorder Hutchinson-Guilford Progeria. Though lamin is a nuclear envelope protein found in all cell types, specific mutations in lamin can give rise to tissue specific diseases such as the neuropathy, Charcot-Marie-Tooth disease (CMT), and muscular dystrophies such as Emery-Dreifuss Muscular Dystrophy (EDMD). As mutant forms of the lamin protein aggregate in these diseases, we tested what mechanism mediates the degradation of lamin during aging. Using the fruit fly Drosophila melanogaster model system, we find that during aging wildtype lamin is targeted for degradation by autophagy through an interaction with the p38 MAPK (p38Kb) and the co-chaperon starvin (stv, BAG-3 in mammalian systems). Interestingly, p38Kb is a regulator of aging and lifespan in flies, and we find that loss of p38Kb leads to accumulation of lamin and abnormally shaped nuclei with chromosomal leakage. We next tested if disease-causing mutations make lamin more aggregate prone, alter its ability to be targeted for degradation, and are associated with neuromuscular dysfunction. We find that different lamin mutations result in altered ratios of lamin protein species, in particular between the non-franesylated and farnesylated forms. In addition, we find differences in the aggregation of the different lamin mutant forms. Finally, we find that expression of these mutant forms results in impaired locomotor functions.

106 **Novel molecular mechanisms in the reactivation and asymmetric cell division of Drosophila neural stem cells** Mahekta Gujar, Yang O Gao, Jiaen O Lin, Ye Sing O Tan, Hongyan O Wang Neuroscience and Behavioural Disorders (NBD), Duke-NUS Medical School

Neural stem cells (NSCs) have the ability to be reactivated from a reversible quiescent state, self-renew and undergo differentiation via asymmetric division. Dysregulation in the balance between quiescence, self-renewal and differentiation can lead to several neurodevelopmental disorders. Here, we demonstrate novel roles of two critical Golgi proteins, Arf1 and its guanine-nucleotide exchange factor (GEF) Sec71 in regulating *Drosophila* quiescent NSC (qNSCs) reactivation and NSC asymmetric division. In *Drosophila*, qNSCs extend a primary protrusion, which is a hallmark of qNSCs. We have unraveled that qNSC protrusions can be regenerated upon injury. This regeneration relies on the Golgi apparatus which acts as the major acentrosomal microtubule-organizing centre in qNSCs. Furthermore, the Golgi-resident GTPase Arf1 and its GEF Sec71 promote NSC reactivation and regeneration via a novel Patronin-Arf1/Sec71-Msps pathway through the regulation of microtubule growth and NSC reactivation. Interestingly, in contrast to its role in regulating non-centrosomal microtubule growth in quiescent neuroblasts we find that Arf1 and Sec71 can regulate neuroblast polarity independent of its known function in microtubules in quiescent neuroblasts. Here, we show that the Golgi proteins Arf1 and ARFGEF2/Sec71 control asymmetric division of *Drosophila* NSCs by physically anchoring myosin II regulatory light chain, Sqh, to the NSC cortex. Arf1 can physically associate with Sqh and Vibrator, a type I PITP that stimulates phospholipid PI4K activity for PI(4)P production. Further, Arf1 and Sec71 are required for PI(4)P localization to the cell cortex of neuroblasts. Our data provides the first evidence that the Golgi proteins Arf1 and its GEF Sec71 can regulate promote NSC reactivation and regeneration via the regulation of microtubule growth and further neuroblast polarity and asymmetric division through phospholipid-dependent non-muscle myosin II cortical localization.

107 **A genetically-encoded method for *in vivo* tagging and tracing of lipids from cell-to-cell** Victor Girard[1], Sebastian Sorge[1], Clare Newell[1], Ian Gilmore[2], Alex P. Gould[1] [1]The Francis Crick Institute, [2]NiCE-MSI, National Physical Laboratory

Lipid transport between cells and tissues is important in health and disease. In both mammals and insects, lipids are transported between tissues via circulating lipoproteins. More local lipid transport also occurs between cells within the same tissue. For example, from neurons to glia during excitotoxicity, inflammation, oxidative stress and models of neurodegeneration. Direct evidence for lipid transfer from neurons-to-glia mostly derives from *in vitro* co-culture models as current *in vivo* methods are unable to tag fatty acids specifically within a single cell type. To overcome this limitation, we developed *conditional cyclopropane fatty acyl tagging* (cFAT). cFAT is a bioorthogonal genetically-encoded method for tagging the monounsaturated fatty acyl chains of phospholipids with a 14 dalton cyclopropane group detectable via mass spectrometry. As proof-of-concept in *Drosophila*, we validate the cFAT method, first in S2 cells and then in live animals using the GAL4/UAS system. We find that cFAT activity is non-toxic and does not interfere with growth or development. cFAT can be used to trace inter-organ FA transport *in vivo* from fat body cells or gut enterocytes into the hemolymph and then on to remote tissues such as the CNS. We also demonstrate how cFAT can be combined with micron resolution mass spectrometry imaging to monitor fatty acid transport between cell types within the CNS. Together, these findings show that cFAT is a powerful method for tracing lipid transport from cell-to-cell that has many potential applications in biomedical research, drug screening and diagnostics.

108 **Chromatin Loops as Regulatory Mechanisms for CAM Gene Expression in Drosophila Neural Development** Xiao Li[1], Jie Hu[1], Michael Levine[1], Maksim Erokhin[2], Darya Chetverina[2] [1]Princeton University, [2]Russian Academy of Sciences

Genes encoding Ccell adhesion molecules (CAMs) are essential for establishing neural circuitry, relying on precise transcriptional regulation for proper developmental outcomes. In our study, we applied the Micro-C technique to visualize the 3D genome organization in Drosophila brains, uncovering an intricate array of chromatin loops that span genomic distances from kilobases to megabases. Remarkably, a substantial proportion of these loops are associated with key CAM genes, such as Dprs, DIPs, Sides, and Beats. Additionally, many CAM genes are connected by these loops, highlighting chromatin architecture's critical role in CAM gene regulation. These loops appear to be instrumental in enabling the spatial and temporal control of gene expression essential for neural development. Some loop anchors enable ultra-long-range interactions crucial for sustaining CAM gene expression across developmental stages. Moreover, mutations in potential looping factors such as GAF and CG11504 disrupt specific 3D architectures, pointing to a combinatorial "code" of regulatory elements that stabilizes essential chromatin interactions. These findings underscore the role of chromatin loops in maintaining gene expression patterns vital for neural development. This work advances our understanding of how 3D genome organization influences CAM gene regulation, providing new insights into the chromatin-based mechanisms of neural circuit assembly in the Drosophila brain.

109 **Distinct input-specific mechanisms enable presynaptic homeostatic plasticity** Dion Dickman[1], Chun Chien[2], Kaikai He[2], Sarah Perry[2], Lisa Tchichkan[2] [1]Neurobiology, University of Southern California, [2]USC

Synapses are endowed with the flexibility to change through experience, but must be sufficiently stable to last a lifetime. This tension is illustrated at the *Drosophila* neuromuscular junction (NMJ), where two motor inputs that differ in structural and functional properties co-innervate most muscles to coordinate locomotion. To stabilize NMJ activity, motor neurons augment neurotransmitter release following diminished postsynaptic glutamate receptor functionality, termed presynaptic homeostatic potentiation (PHP). How these distinct inputs contribute to PHP plasticity remains enigmatic. We have used a botulinum neurotoxin to selectively silence each input and resolve their roles in PHP, demonstrating that PHP is input-specific: Chronic (genetic) PHP selectively targets the tonic MN-Ib, where active zone remodeling enhances Ca^{2+} influx to promote increased glutamate release. In contrast, acute (pharmacological) PHP selectively increases vesicle pools to potentiate phasic MN-Is. Thus, distinct homeostatic modulations in active zone nanoarchitecture, vesicle pools, and Ca^{2+} influx collaborate to enable input-specific PHP expression.

110 **Homeodomain Proteins in Newborn Neurons: Codes for Neuron Type Specification and Expansion** Chundi Xu[1], Peter Newstein[2], Rishi Sastry[2], Chris Doe[1] [1]HHMI, University of Oregon, [2]University of Oregon

Our ability to perceive and respond to the world depends on a vast diversity of neuron types, each characterized by unique morphology and functional properties. Although recent advances in single-cell transcriptional profiling have enabled us to map diverse neuron types across species such as *Drosophila*, mice, and even humans, we still lack a mechanistic understanding of how these neuron types arise during development and expand during evolution. We have identified homeodomain transcription factor (HDTF) codes in newborn neurons that specify the five neuron types (L1-L5) in the *Drosophila* lamina, the first ganglion of the visual system. Specifically, we found that the pan-lamina HDTF, Scro, is essential for lamina progenitors to exit proliferation and differentiate into lamina neurons. Within these newborn lamina neurons, distinct HDTFs are expressed in specific subpopulations, acting as transcriptional codes that specify different lamina neuron types: Zfh1 specifies L1 and L3, Dve specifies L2, and Bsh specifies L4 and L5. Moreover, we found that neurons lacking their specific HDTF codes revert to different neuron types, potentially representing ancestral neuron types: the loss of Dve causes L2 neurons to revert to the L3 neuron type, while the loss of Bsh causes L4 and L5 neurons to revert to L3 and L1 neuron types, respectively. Additionally, we found that HDTF codes can be modified by Notch signaling in newborn neurons, allowing a single HDTF to specify two different neuron types depending on the presence or absence of Notch signaling. HDTFs are evolutionarily conserved and broadly expressed in brains across species, and we found that neurons in the mouse retina also express neuron type-specific HDTFs. Based on these findings, we propose a model in which distinct HDTFs in newborn neurons act as transcriptional codes that specify unique neuron types during development and have contributed to the expansion of neuron types during evolution.

111 **Ca^{2+} dynamics regulate cellular identity and cell cycle progression in neural stem cell lineages** Bernice C Lin[1], Isabella R Maag[1], Hannah Christman[2], Asher Swan Adams[1], Jillian Wynne[1], Beverly J Piggott[1] [1]Division of Biological Sciences, University of Montana, [2]University of Montana

The role of calcium (Ca^{2+}) in stem cell identity and function is widely recognized as significant, yet the underlying mechanisms remain poorly resolved. Various Ca^{2+} dynamics, such as sustained, transient, or oscillatory changes in Ca^{2+} levels, can enact distinct cellular behaviors and trigger transcriptional changes. Asymmetric Cell Division (ACD) is a widely conserved mechanism by which stem cells can expand and diversify cellular populations. Work in flies has defined fundamental principles of ACD broadly conserved across cell types, but the role of Ca^{2+} in this context is limited. Utilizing a genetically encoded Ca^{2+} sensor, my lab has resolved cell-type specific Ca^{2+} dynamics of asymmetrically dividing, neuroblast (NB) lineages, within an intact brain. Within the type II NB lineage, we found that baseline cytoplasmic Ca^{2+} levels increased in cells as they become more differentiated, while NBs exhibited very low cytoplasmic Ca^{2+} levels. Strikingly, inducing high cytoplasmic Ca^{2+} levels in the type II NB lineage through RNAi knockdown of SERCA (sarcoendoplasmic reticulum calcium ATPase) was sufficient to shift the identity of type II NB lineages into a type I NB lineage fate. These reprogrammed "type I like-NB lineages" displayed protein expression markers and generated progeny consistent with a type I NB identity. To determine if Ca^{2+} regulation was important for NB proliferation, we performed a knockdown screen of multiple calcium regulatory proteins and discovered many of them, including SERCA, compromised proliferation. Notably, only SERCA caused an identity shift. Our experiments have begun to unravel the mechanisms by which type II NB orchestrate Ca^{2+} dynamics to maintain identity and proliferation. Our data indicates that different Ca^{2+} sources distinctly regulate NB identity and cell cycle progression. Intracellular Ca^{2+} gradients are also found in skin where mutations in human SERCA2 causes the blistering skin disorder, Darier's disease. Intriguingly, Darier's disease patients are 4x more likely to be diagnosed with bipolar disorder and 2.5x more likely to be diagnosed with Schizophrenia indicating that Ca^{2+} regulation of neural development may be conserved to humans. Our work will define the mechanistic basis for Ca^{2+} regulation of stem cell identity and function and may identify new targets for therapeutic interventions of developmental disorders.

112 **How flexibility in stem cell biology drives interspecies neuronal diversity** Sam Swank, Ellie Heckscher MGCB, University of Chicago

Neuronal diversity is crucial for animal behaviors. An organism's neuronal population is diversified by the collective activity of three fundamental mechanisms: spatial patterning, temporal patterning, and Notch signaling. Different species possess distinct anatomy and demonstrate unique behaviors which require neuronal differences to control them. Yet, how neural patterning mechanisms are flexible to produce interspecies diversity is entirely unknown. This is critical to understanding of how development is flexible to generate new neuron types. Prior comparative research highlighted conservation of cell types, mainly for lack of a tractable entry point to study new neuron types in interspecies comparisons. We use the fruit fly (*Drosophila melanogaster*) for comparison because its behavior and anatomy are well studied. Additionally, the relationship between neural patterning and single neuron fates are known. Scuttle flies (*Megaselia abdita* and *M. scalaris*) display unique behaviors and possesses additional muscles, raising the question of how the CNS is adapted. *Megaselia* and *Drosophila* possess the same number of motor neurons. Still, in *Megaselia* we identified an extra, previously undescribed neuron type, suggesting that new cell types deep within the CNS could play a role in behavioral lability. The *Megaselia*-specific neuron expresses Even-skipped (Eve), a highly conserved, essential transcription factor that dictates neuronal morphology and function from insects to vertebrates. This finding is shocking because prior studies of ~20 species across >500MY of evolution found no differences in Eve expression. My data show novel neurons arise early in development, leading to three models for how novel neurons could arise in *Megaselia*. First, there may be differences in the neural stem cell patterning (in terms of neural stem cell identity or number). Alternatively, there could be a *Megaselia*-specific extension of an early temporal patterning window (heterochrony). Finally, the relationship between Notch signaling and neuron fate (e.g., Eve expression) may be disrupted in *Megaselia*. We will report on our progress in distinguishing these three models.

113 ***mir-279/996* controls sensory organ development and behavior via discrete targets** Binglong Zhang, Eric Lai, Binglong Zhang Development, MSKCC

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Abstract:
microRNAs (miRNAs) are ~22 nucleotide regulatory RNAs that are proposed to repress extensive target cohorts, since miRNAs often have hundreds of conserved target sites across the transcriptome. However, in the 30 years after the Nobel prize-winning work by Ambros and Ruvkun on the first miRNA-target pair, relatively few miRNAs have proven essential for in vivo development, and even fewer critical individual targets are known. We found that the *mir-279/996* cluster, which generates two similar miRNAs, controls the development and function of several classes of sensory organs. This is particularly overt in Johnston›s organ (JO), the primary proprioceptive and auditory structure of insects. Loss of *mir-279/996* ablates normal JO development, with loss of scolopale cells and ectopic neurons, yielding completely deaf animals. We showed that miR-279/996 autonomously suppress neural fate in non-neuronal cells, and found several miR-279/996 targets that are dose-sensitive for *mir-279/996* phenotypes. Interestingly, all of these encode neural-restricted regulatory factors. To directly evaluate the causality of their miRNA regulation for JO phenotypes, we used CRISPR/Cas9 to construct a panel of mutants bearing specific mutations in miR-279/996 target sites. Excitingly, a subset of miRNA target site mutants exhibit defective JO development, and this is exacerbated in certain double and triple mutant combinations. Ongoing efforts will complete the set of genetic combinations, which we are analyzed using JO cytology and electrophysiology. This study will be one of the first to define a discrete set of miRNA target sites, that are both directly and critically responsible for normal developmental patterning.

114 **miR-137 targets Myc to regulate Development and Tumor Growth in *Drosophila* eye model** Radhika Padma[1], Anuradha Chimata[1], Arushi Rai[1], Manivannan Subramanian[1], Madhuri Kango-Singh[1,2,3,4], Amit Singh[1,2,3,4,5] [1]Biology, University of Dayton, [2]Premedical Program, University of Dayton, [3]Center for Tissue Regeneration and Engineering at Dayton (TREND), University of Dayton, [4]Integrative Science and Engineering (ISE), University of Dayton, [5]Center for Genomic Advocacy (TCGA), Indiana State University

During organogenesis, the regulation of gene expression controls the fundamental cellular processes like cell proliferation, differentiation, and cell death. Among many gene regulatory molecules, miRNA(s), a class of small non-coding RNAs, regulate more than one target mRNA expression during development. To understand the molecular-genetic mechanism(s) regulating eye development, we employed a forward genetic screen to identify the miRNA involved in controlling patterning and growth in the *Drosophila* eye. We identified miR-137 whose gain-of-function (GOF) in developing eye results in reduced eye phenotype and loss-of-function results in enlarged eye phenotype. We found that reduced eye phenotype is accompanied with reduced expression domains of retinal determination and differentiation markers, and increased negative regulators of eye development like Wingless (Wg) and Homothorax (Hth). In the developing eye, Wg is known to block the progression of a synchronous wave of differentiation referred to as the Morphogenetic furrow (MF). The GOF of miR137 significantly downregulates the expression of *dpp*-lacZ, an MF marker. Using bioinformatic approaches, followed by genetic experiments, we identified Myc as a target of miR-137 as the GOF of Myc can rescue reduced eye phenotype of miR-137 GOF or vice versa. Our data suggest a new role of miR-137 in targeting Myc in the developing eye to determine eye size by regulating retinal- determination, -differentiation, and cellular homeostasis. We tested GOF of miR-137 in RasV12 , scrib RNAi background, an established tumor model for oncogenic cooperation that results in neoplastic tumors. The GOF of miR-137 show a significant rescue of tumor phenotype in the eye along with a significant reduction in Myc levels. Our studies shed light on the role of miR-137 in tissue homeostasis, growth regulation, and development.

115 **Intron-mediated delays in gene expression controls ~24-hour circadian rhythms** Swathi Yadlapalli, Ye Yuan University of Michigan

Introns are traditionally viewed as primarily influencing alternative splicing and protein diversity. However, our research uncovers a novel function for a specific intron in a core clock mRNA: it introduces a critical time delay in gene expression, critical for generating ~24-hour circadian rhythms. Using RNA Fluorescent In Situ Hybridization and RNA-sequencing, we found that ~50% of *timeless* mRNAs, a Drosophila core clock mRNA, remain nuclear due to a unique intron retention event. This intron is spliced out post-transcriptionally near nuclear speckles, inducing a vital 2-hour delay in circadian rhythms. CRISPR-mediated deletion of this intron results in rapid mRNA accumulation in the cytoplasm, accelerated TIM protein synthesis, and a shortened circadian cycle to ~22 hours. Furthermore, this intron, lacking a traditional branch point, when inserted into reporter minigene transcripts, ensures nuclear retention of any mRNA in both Drosophila neurons and human U2OS cells—a reversible process by adding a lariat branch point. Additionally, we show that the RNA-binding protein Qkr58E-2, homologous to mammalian Sam68, is essential for activating this splicing; its knockdown leads to increased nuclear retention of *timeless* mRNA, decreased TIM protein levels, and disrupted circadian rhythms. These findings reveal a novel rate-limiting mechanism within circadian clocks, highlighting the significant regulatory role of intron splicing dynamics in gene expression. Our study highlights introns as critical regulatory elements in timing gene expression, suggesting their broader impact on biological processes beyond circadian rhythms.

116 **Alcohol alters gene expression in GABA neurons via the mondo signaling pathway** Collin Merrill[1], Alexandra Seguin[2], Emily Nickel[2], Aylin R Rodan[2,3,4,5], Adrian Rothenfluh[1,2,4,6] [1]Psychiatry, University of Utah, [2]Molecular Medicine Program, University of Utah, [3]Internal Medicine, University of Utah, [4]Human Genetics, University of Utah, [5]Medical Service, Veterans Affairs Salt Lake City Health Care System, [6]Neurobiology, University of Utah

In Drosophila, like in humans, GABA neurons are present throughout the brain and are important components of alcohol-induced behaviors. Exposure to low doses of alcohol causes GABAergic hyperactivity, which may alter the chromatin landscape in a manner similar to the activity-induced changes in chromatin accessibility observed in other neuron populations. Tolerance to alcohol requires neuronal plasticity, which is caused by changes in chromatin accessibility, altered gene expression, and dysregulated neuronal activity. To understand how alcohol alters chromatin-mediated gene regulation in GABA neurons, we used assay for transposase-accessible chromatin by sequencing (ATAC-seq) to examine the chromatin landscape in GABA neurons after exposure to acute, low-dose alcohol. We observed that alcohol caused many chromatin regions became more closed in GABA neurons and that many of these regions were associated with genes belonging to the insulin receptor signaling pathway. To examine whether insulin receptor signaling within GABA neurons is involved in alcohol-mediated sedation and the development of tolerance, we expressed constitutively active insulin receptors within GABA neurons and measured alcohol-induced sedation and tolerance. Activation of insulin receptor signaling caused sensitivity to sedation and increased tolerance. To further elucidate how alcohol affects chromatin-mediated regulation of genes associated with insulin receptor signaling, we asked whether the chromatin regions associated with insulin receptor signaling genes that became more closed by alcohol contain common motifs. These closed chromatin regions were enriched for binding sites for the transcription factor, mondo., We used mondo RNA to test whether mondo is involved in alcohol-induced sedation and tolerance and observed resistance to alcohol-induced sedation and decreased tolerance. We then examined whether mondo transcription factor binding sites are present within the insulin receptor signaling-associated chromatin regions that became closed after ethanol exposure. This analysis identified several potential mondo target genes, including *mustard* (*mtd*) and several genes corresponding to vesicular ATPases (v-ATPases). To determine if ethanol exposure changes the expression of mondo target genes, we performed quantitative PCR on nuclei from GABAergic neurons after the development of tolerance. We observed decreased expression of *mondo*, its binding partner *bigmax*, *mtd*, *CG3764*, *stnB*, *Vha26*, and *VhaSFD*. Together, these results suggest that alcohol acts through insulin receptor signaling to alter mondo transcription factor activity, which subsequently decreases v-ATPase and accessory protein expression, which may underlie the development of tolerance to alcohol.

117 **A malleable pipsqueak amyloid controls polycomb complex function in Drosophila** Camila Behrensen[1], Matthew Christenson[1], Kaili Li[1], Jeff Lange[1], Paulo Leal[1], Kaelan Brenan[1], Dai Tsuchiya[1], Zulin Yu[1], Julia Zeitlinger[1], Ruben Hervas Milan[2], Kausik Si[1] [1]Stowers Institute for Medical Research, [2]School of Biomedical Sciences, Hong Kong University

The maintenance of cell identity relies on the precise regulation of gene expression. Polycomb group proteins (PcG), recruited by DNA-binding proteins to their gene targets, establish and maintain a repressive transcriptional state, with mechanisms not entirely clear and being intensely investigated. Here, we discovered that Drosophila Pipsqueak (Psq), one of the PcG-recruiting DNA-binding proteins, forms prion-like aggregations on chromatin and remains associated with mitotic chromosomes in embryos. The *psq* gene encodes a long (PsqL) and a short (PsqS) protein isoforms. Both are enriched on GA-rich sequences, but only PsqL forms fibrous aggregates. Structure analysis by cryo-EM reveals that these PsqL fibers purified from embryos are amyloids. Remarkably, the PsqL fibers induced from Psq monomers purified from embryos in the presence of GA-rich oligos *in vitro* process a structure identical to that of endogenously purified amyloids, implying that DNA binding of Psq is inducive to amyloid formation *in vivo*. Our data also suggest that PsqL polymers specifically interact with the Pc protein, a core component of the PRC1 complex, to regulate the monoubiquitylation of H2AK118 by PRC1. Interestingly, deleting only the prion-like domain or the DNA-binding domain of Psq causes more severe developmental defects than deleting Psq, suggesting that the two mutant proteins retain molecular functions that interfere with normal development, possibly by sequestering PcG. As amyloid structures are known for their extraordinary stability and self-propagating ability, our findings on Psq's role in PcG regulation propose an attractive alternative mechanism for the inheritance of epigenetic states in dividing cells.

118 **Probing the boundaries of *cis* element flexibility at the *Drosophila* histone locus** Lauren J Hodkinson[1,2], Julia Gross[3], Casey A Schmidt[4], Annalise Weber[3], Leila E Rieder[1] [1]Department of Biology, Emory University, [2]Department of Molecular Biology, Cell Biology, and Biochemistry, Brown University, [3]Emory University, [4]Department of Biology, Lafayette College

Transcription factors have access to a subset of their possible binding sites at any given time and genomes themselves sport many *cis* elements similar in sequence. Some transcription factors can carry out distinct functions in different contexts by integrating cues from specific *cis* element structure, genomic context, and local chromatin characteristics. We used the repetitive *Drosophila* histone gene locus to probe the boundaries of *cis* element flexibility. The *D. melanogaster* genome carries a single "histone locus" with ~100 tandemly repeated arrays, each including the 5 replication-dependent histone genes. We discovered that the 100 histone gene arrays are nearly identical in sequence except for the length of a GA-repeat within the *H3/H4* promoter. This GA-repeat is targeted by the transcription factor CLAMP to promote histone gene regulation. Therefore, we hypothesized that the GA-repeat length impacts CLAMP occupancy and regulation of specific histone genes. We mined embryo ChIP-seq data from three competitive GA-binding proteins: CLAMP, GAGA Factor (GAF), and Pipsqueak (Psq), for occupancy at the histone locus. All three proteins target the *H3/H4* GA-repeat when we analyze the data in bulk. To determine if the GA-repeat length leads to differential factor occupancy, we developed a novel pipeline to extract relevant ChIP-seq reads that span the GA-repeat variability. Surprisingly, there is no correlation between factor occupancy and GA-repeat length for any factor. Interestingly, we also observe that the GA-repeats are not well conserved, yet CLAMP still interacts with the histone genes in those species, implying that CLAMP is amenable to small differences in *cis* element structure. We therefore probed the boundaries of *cis* element flexibility using a transgenic approach. We engineered flies to carry a transgenic histone array in which we replaced the natural GA-repeat with X-linked CLAMP-recruiting elements. Interestingly, these X-linked CLAMP binding sites did not functionally substitute the GA-repeat when placed in the *H3/H4* promoter and prompted CLAMP to recruit X-chromosome specific factors to the autosomal transgene. Overall, our results suggest that the *cis* element length, but not sequence, is flexible. Future studies should interrogate additional *cis* elements or factors that may work in combination to impact histone gene regulation and development of methods to profile histone transcripts from individual genes.

119 **Aggregation and DNA binding of Dorsal/NF-kappaB in early embryos** Sadia Siddika Dima[1], Gregory T. Reeves[1,2] [1]Department of Chemical Engineering, Texas A&M University, [2]Faculty in Genetics and Genomics, Texas A&M University

The mechanism by which transcription factors (TFs) regulate gene expression, a process crucial for the development and maintenance of an organism, remains puzzling despite decades of research. Recent advances propose that TFs form dynamic, possibly phase-separated hubs that contribute to transcriptional regulation. However, the mechanisms connecting hub formation with transcriptional regulation remain poorly understood. To address this gap, we measured biophysical properties of the TF Dorsal (Dl), which forms a gradient to pattern the dorsal-ventral (DV) axis of the early *Drosophila* embryo.

Previous work using fluorescent imaging of live and fixed embryos indicated that the Dl gradient has a Gaussian-like shape with the highest level of Dl present on the ventral side of the embryo. The level of Dl oscillates in a progressively increasing saw-tooth pattern during blastoderm nuclear cycles (ncs) 10 to 14. The spatiotemporal variation of Dl levels ensures the accurate spatial borders and dynamic pattern of target gene expression. Here we use Raster Image Correlation Spectroscopy (RICS), a type of scanning Fluorescence Correlation Spectroscopy, and single particle tracking in live embryos to quantify absolute concentrations of three sub-populations of Dl, each with distinct diffusivities and binding properties, along the DV axis from nc 10 to 14. We show that, while the absolute nuclear concentration of Dl increases over time, the fraction of Dl bound to DNA decreases over time. The DNA binding cannot be explained by a simple dose response relationship between free and bound concentrations, implying the existence of a mechanism beyond simple TF/DNA binding. The results from all the experiments encompassing different length and time scales suggest the presence of slowly moving clusters of Dl in addition to the expected populations of freely mobile Dl and DNA bound Dl. The data suggest that the clusters form only above a threshold concentration, a phenomenon typically associated with phase-separation. The formation of similar mobile clusters has been previously observed in other TFs, such as Bicoid, and has been proposed to provide an efficient search strategy for regulatory regions. We also found that the clusters bind the DNA transiently. These findings enhance our understanding of the mechanism of transcriptional interpretation of the Dl gradient and are expected to generalize to other TF/DNA interactions.

120 **Cell cycle-regulated transcriptional pausing controls replication-dependent *Drosophila* histone gene expression** Mark S. Geisler[1], James P. Kemp[2], William F. Marzluff[2,3,4], Robert J. Duronio[2,4,5] [1]Curriculum in Genetics and Molecular Biology, University of North Carolina - Chapel Hill, [2]Integrative Program for Biological and Genome Sciences, University of North Carolina - Chapel Hill, [3]Department of Biochemistry and Biophysics, University of North Carolina - Chapel Hill, [4]Department of Biology, University of North Carolina - Chapel Hill, [5]Department of Genetics, University of North Carolina - Chapel Hill

Replication of a eukaryotic genome requires the production of hundreds of millions of histone proteins to package the newly synthesized DNA. To meet this large demand for histone production, eukaryotes coordinate high level expression of replication-dependent (RD) histone gene clusters during S-phase of the cell division cycle. Transcription and processing of RD-histone RNA occurs within a biomolecular condensate conserved from *Drosophila* to humans that assembles at RD-histone genes called the Histone Locus Body (HLB). Expression of RD-histone genes is thought to be activated during S phase by Cyclin E/CDK2 phosphorylation of the HLB scaffold protein, MXC (*Drosophila*)/NPAT (human), but the step(s) of transcriptional control regulated by Cyclin E/Cdk2 are unknown. We used fluorescently tagged RNA polymerase II (RNAPII) subunits and fluorescent in situ hybridization (FISH) to core histone RNAs to monitor RD-histone transcription dynamics in HLBs in *Drosophila* imaginal discs. We found that RNAPII is present in the HLB throughout the cell cycle, not just in S-phase, and that some HLBs with RNAPII did not transcribe RD-histone RNA, indicating recruitment of RNAPII to the HLB does not directly lead to transcription. We show relative levels of RNAPII correlate with the amount of MXC in the HLB suggesting they are co-recruited to the HLB. To test if RD histone transcription is regulated through promoter proximal pausing, we designed a FISH probe to the 5' portion of the core RD-histone RNAs, including the 5' UTR, and to the 3' portion, downstream of any known RNAPII pause sites in the RD histone genes. When performing RNA-FISH with both the 5' and 3' probes, we observe both 5' and 3' signal in HLBs in S-phase cells, corresponding to elongating RD histone gene transcripts. However, in both G1 and G2 cells that do not contain phosphorylated MXC, we observe only 5' histone RNA signal, indicative of paused transcripts. We further showed that transcriptional initiation of the RD-histone genes can be independent of Cyclin E as HLBs in Cyclin E null embryos express only 5' nascent RD histone RNA signal. Thus, Cyclin E/CDK2 phosphorylation of MXC is required to release promoter proximal paused RNAPII during S-phase, but not to initiate transcription of the RD histone genes, which can occur outside of S-phase. These results provide a new paradigm for how RD-histone mRNA production is coupled to S phase. This work also demonstrate that the HLB and RD-histone genes can serve as a visual readout for studying transcriptional dynamics that can be quickly and cheaply assessed without the need for genomic experiments.

121 **The Contribution of Genome Structural Variants to Complex Trait Variation in *Drosophila melanogaster*** Trevor D Millar[1,2], Mahul Chakraborty[2] [1]Interdisciplinary Graduate Program in Genetics and Genomics, Texas A&M University, [2]Department of Biology, Texas A&M University

Genome structural variants (SVs) may account for a portion of heritable variation in complex traits, with broad implications for human diseases, plant and animal breeding, and adaptive evolution. In particular, large deletions, duplications, insertions and translocations are hypothesized to modify the regulatory landscape by disrupting regulatory regions or by introducing novel regulatory elements. Despite this, current research on genetic variants underlying complex trait variation has primarily focused on single nucleotide polymorphisms (SNPs) and small indels, which explain only a fraction of the variation in these traits. Structural variants remain underexplored due to challenges with SV discovery and genotyping. These limitations can be mitigated by genotyping SVs with a pangenome reference graph that captures a broader SV spectrum, providing a robust framework for comprehensive genotyping.

To evaluate the contribution of SVs to complex trait variation, we conducted comprehensive SV genotyping within the Drosophila Genetic Reference Panel (DGRP), a community resource for quantitative trait mapping, using a pangenome graph created from 32 highly contiguous genome assemblies. We applied a regression framework that enables direct comparison of the effects of SNPs, indels, and SVs on phenotypic variation, quantifying their relative contributions to trait variance and thereby highlighting the role of structural variation alongside smaller variants. To elucidate the molecular mechanisms of SVs underlying expression variation, we overlaid annotated ATAC-Seq peaks with our genotyped SVs, identifying candidates that influence regulatory sequences. The integration of ATAC-Seq with SV data highlights how SVs disrupt or create regulatory regions, potentially affecting transcription factor binding sites and altering gene expression.

Finally, we compared allele frequencies of these SVs across the DGRP and other populations, identifying putatively adaptive SV alleles that segregate at high frequencies. These findings suggest that these SVs may be under selection, potentially contributing to population-specific trait variation in D. melanogaster.

122 **Characterizing the pupal development of wave one ovarian follicles in *D. melanogaster*** Yunpeng Wayne Fu[1], Allan C Spradling[2] [1]Embryology, Johns Hopkins University, [2]Embryology, Carnegie Institute for Science

Mouse oogenesis happens in two temporally distinct waves. While all primordial germ cells (PGCs) immediately form germline cysts after reaching the gonad around E10.5, about 5% generate wave one follicles that retain bipotential granulosa cells, reside in the medulla, and develop without delay to the preantral stage. In contrast, cortical follicles, termed wave two, acquire an epithelial-derived granulosa covering, form an arrested ovarian reserve and only mature in small cohorts throughout adulthood. We have examined two potentially similar subgroups of *D. melanogaster* follicles. At least 60% of PGCs begin to develop at the end of L3, rather than forming germline stem cells (GSCs) supporting oogenesis throughout adulthood. Adult oogenesis via GSCs is extensively characterized, but much less is known about wave one-like directly developing PGCs. Using sparse lineage tracing of more than 3,000 individual L3 PGCs starting at different developmental time points, we analyzed Drosophila wave one and early GSC-based follicle production. Our findings clarified the precise numbers and behavior of both groups, including more early PGC symmetric divisions than previously believed. About 50% of wave 1 follicles differ from GSC-generated follicles in their developmental program and undergo apoptosis prior to metamorphosis. The other 50% generate around 120 of the earliest follicles present in the ovary at eclosion. Our results suggest that follicle development in distinct waves, which likely also occurs in humans, is a conserved feature of oogenesis.

123 **Mild chronic cold improves female germline stem cell maintenance despite activation of transposable elements in the germline** Ana Caroline Gandara[1,2], Daniela Drummond-Barbosa[2] [1]Morgridge Institute for Research, [2]University of Wisconsin-Madison

Temperature influences fertility in many organisms; however, the mechanisms underlying how suboptimal temperatures affect adult gamete production and quality remain largely unknown. Our previously work showed that germline stem cell (GSC) numbers and oocyte quality remain higher over time in *Drosophila melanogaster* females maintained at 18°C compared to controls at 25°C. On the other hand, 29°C decreases hatching rates, while not affecting GSC numbers. To investigate the underlying mechanisms, we compared the ovarian transcriptome of females after five days at 18, 25, or 29°C. We found that 18°C modulated three times as many genes as 29°C, showing that there is an active physiological response to cold, involving upregulation of many genes. High temperatures have been previously associated with activation of transposable elements (TE), in agreement with our observations that many TEs are significantly increased in the ovaries at 29°C. Remarkably, however, a larger number of distinct TEs were also upregulated at 18°C, representing the first example to our knowledge of TE activation by cold in animals, adding to the rare examples in plants. Also, the differential TE activation at 18°C versus 29°C suggests that distinct mechanisms are at play. Activation of TEs is typically associated with genome instability as they cause double DNA breaks. Indeed, we observed an increased fraction of GSCs stained for γH2Av (a common marker for DNA breaks) at 18°C, compared to 25°C. These findings appeared paradoxical considering the improved GSC numbers at 18°C. Interestingly, our transcriptomics data showed that R2 is the most upregulated TE in ovaries at 18°C. R2 is a retrotransposon that has recently been shown to be required in the male germline to maintain rDNA copy number and GSC maintenance. We therefore examined the nucleoli of GSCs using a nucleolar marker, fibrillarin. Both area size and intensity of fibrillarin were increased in GSCs at 18°C, compared to 25°C. rRNA transcription in female GSCs affects the expression of proteins that regulate cell fate within the germline. Disruption of rRNA biogenesis results in reduced levels of Mad, that represses GSC differentiation, for example. With that, we measured pMad intensity in GSCs nuclei and found that cold also increases pMad staining. Based on these findings, we hypothesize that GSC stemness is higher at 18°C in part through R2 activation to enhance nucleolar function and niche signaling. Funding: NIH R35 GM140857.

124 **Ecdysone signaling and BMP signaling converge to regulate ovarian germline stem cell maintenance and differentiation** Alexandria Warren[1], Lauren Jung[1], Changhong Yin[2], Weihua Huang[2], Zhipeng Sun[3], Todd Nystul[4], Elizabeth Ables[1] [1]Biology, East Carolina University, [2]Pathology and Laboratory Medicine, Brody School of Medicine, East Carolina University, [3]National Key Laboratory of Green Pesticide, South China Agricultural University, [4]Anatomy and OB/Gyn, University of California San Francisco

Ovarian germline stem cells (GSCs) are essential to maintain oocyte production and fertility in adult females. GSCs depend on both steroid hormones and local paracrine signals to regulate their proliferative ability and long-term maintenance. Signaling by Bone Morphogenetic Protein (BMP) ligands via the receptors Thickveins (Tkv), Saxophone (Sax), and Punt (Put) repress transcription of the differentiation gene, *bag of marbles* (*bam*), allowing for GSC self-renewal. Concurrently, the steroid hormone ecdysone promotes BMP signaling in GSCs, but the molecular mechanisms by which this regulation is achieved are unknown. Ecdysone regulates the transcription of target genes through two nuclear receptors, Ecdysone Receptor (EcR) and Ultraspiracle (Usp). To elucidate the role of EcR in germ cells, we built germ-line compatible tools to manipulate levels of *EcR*. Over-expression of *EcR* in germ cells blocked germ cell differentiation, resulting in masses of undifferentiated cells phenotypically resembling *bam* loss-of-function and *Tkv* constitutive activation. To identify nodes of functional similarity, we compared gene expression in these three genetic models using single cell RNA sequencing. Here, we provide evidence that *EcR*-expressing germ cells most closely resemble germ cells with constitutive activation of *Tkv*, with only a few genes differentially expressed between the two models. We found no differential expression between *EcR*-expressing and *Tkv*-expressing germ cells in either BMP signaling components or ecdysone-responsive genes, and both lead to repression of *bam* transcription. We demonstrate that *EcR* over-expression increases *Tkv* expression at the mRNA and protein levels. Finally, comparison between the three genetic models allowed us to separate six independent transcriptional states of undifferentiated cells, identifying novel points of regulation downstream of BMP signaling, ecdysone signaling, or both. We propose that steroid hormones from adjacent escort cells are received by GSCs, resulting in a transcriptional program that converges on BMP signaling in a feedback loop to promote GSC proliferation and regulate differentiation. Together, these data show a strong autonomous role for ecdysone signaling in GSC maintenance and differentiation and reveal putative novel regulators of germline development.

125 **Programmed nuclear pore replacement during *Drosophila* oogenesis** Shruti Venkat[1], Maya Capelson[2], Prashanth Rangan[1] [1]Department of Cell, Developmental, and Regenerative Biology, Icahn School of Medicine at Mount Sinai, [2]Cell and Molecular Biology Program, Department of Biology, San Diego State University

Germ cells possess the remarkable ability to reset their cellular age, thereby initiating new life. The precise molecular mechanism underlying this rejuvenation process remains elusive. The germ cell-derived oocyte supplies a reservoir of "maternal contribution," including organelles and complex macromolecules, which play a pivotal role in initiating embryogenesis. However, it remains unclear if and how the maternal contributions are selected to weed out aged or inappropriate components. Using Drosophila oogenesis as a model system, we found that the nuclear pore complex (NPC)—a maternally provided structure serving as a gateway between the nucleus and cytoplasm—exhibits a unique pattern. In the female germline, NPC levels decrease before oocyte specification and subsequently increase afterward. Results from an RNAi screen indicate a crucial role for the ESCRT-III protein complex and Vps4. These proteins facilitate membrane scission and the removal of NPCs from the nuclear membrane, which are then fed into lysosomes for degradation via the endocytic pathway concurrent with oocyte specification. Depletion of ESCRT-III components or Vps4 in the germline results in the accumulation of NPCs during oocyte specification, ultimately leading to the failure of oocyte specification and extinguishing of early germ cell program at the required timepoint. Concurrent with ESCRT-III mediated NPC degradation, there is a simultaneous rise in nascent transcription of nucleoporins that constitute the NPCs, facilitating the production of new NPCs. These findings indicate a dynamic process in which NPCs in the female germline undergo degradation and subsequent resynthesis of new NPCs for maternal contribution. In contrast, in the male germline, NPCs increase significantly until sperm maturation at which point most of them are eliminated from mature sperm before fertilization. Thus, our work proposes a model in which maternal components, such as NPCs, undergo selective degradation and renewal, effectively resetting them to support the developmental needs of the next generation.

126 **Dafcin, a novel amphipathic peptide with membrane-penetrating ability in the *Drosophila* ovary** Kevin G. Nyberg, Ryder Easterlin, Hamdi K. Kucukengin, Kyung Je Lee, Silpol Dhiantravan, Megan A. Wong, Richard W. Carthew Northwestern University

While most known peptides are processed from larger protein precursors, peptides translated directly from short open reading frames (sORFs) are increasingly being discovered through evolutionary analyses and peptidomics approaches. Here, we describe a novel *Drosophila* peptide translated from a sORF that we have named Dafcin on account of its genomic location within an amplified region in the ovarian follicle cells (i.e., *Drosophila* Amplicons in Follicle Cells). Misannotated as a lncRNA in current *Drosophila* genome annotations, the *Dafcin* gene possesses a short but conserved open reading frame (21 amino acids) that strongly suggests coding potential. Translation of Dafcin was subsequently verified in vivo using a Dafcin-GFP fusion protein. Dafcin is predicted to be an amphipathic alpha-helical peptide, a class of peptides that functions via interactions with lipid bilayers and includes viral fusion peptides and cell-penetrating peptides. Dafcin-GFP is initially expressed in the ovarian follicle cells which surround the oocyte at Stage 10B of oogenesis, prior to nurse cell dumping. Remarkably, Dafcin-GFP also localizes to the oocyte in subsequent stages of oogenesis, suggesting that it is secreted by the follicle cells and imported into the oocyte. This intercellular transport was verified using follicle cell-specific RNAi knockdown of Dafcin-GFP, indicating that Dafcin possesses at least limited membrane-penetrating ability. In the oocyte, Dafcin-GFP localizes inside the yolk granules. Uptake of Dafcin-GFP into the oocyte was blocked in mutants of the low-density lipoprotein receptor Yolkless, indicating that Dafcin-GFP import into the oocyte is largely dependent on receptor-mediated endocytosis. Using quantitative image analysis of *Dafcin* frameshift mutants, mutant yolk granules were found to be significantly larger than those in wildtype flies. Dafcin's predicted peptide structure has notable physico-chemical similarities to the HA fusion peptide of influenza virus, which also enters host cells through endocytosis and functions to induce negative curvature in host lipid bilayer membranes to promote fusion of host and viral membranes. Thus, we hypothesize that Dafcin functions to induce negative curvature in the membranes of yolk granules, ultimately resulting in smaller yolk granules.

127 **Somatic cells non-autonomously control germline incomplete cytokinesis through FGF signaling** Beth Kern, Sam Price, Kari F. Lenhart Biology, Drexel University

Building gametes, egg and sperm, is a complex yet highly conserved process. From *Hydra* to humans, germ cells execute a specialized program: incomplete cytokinesis. To build a robust germline, germ cells divide several times mitotically as they differentiate. In most cell types, mitosis culminates in completion of cytokinesis and abscission, or membrane severing, producing two independent daughter cells. By contrast, germ cells do not complete cytokinesis. Instead, cytokinesis machinery is reorganized to build stable intercellular bridges termed ring canals (RCs) that allow for cytoplasmic sharing and generate interconnected germline cysts. Despite our understanding of the cytoskeletal network surrounding RCs, little is known regarding the dynamics of these proteins across the cyst cell cycle. Using the *Drosophila* male germline, I live imaged reiterative rounds of incomplete cytokinesis and have found two distinct mechanisms of generating RC F-actin. At mitotic exit, each germ cell assembles and arrests an actomyosin contractile ring, generating new germ cell daughters. Interestingly, the RCs generated in the previous round of mitosis must *de-novo* re-polymerize F-actin – resulting in a chain of "new" and "old" RCs linking germ cells within the cyst. Furthermore, I find that maintenance of RC F-actin is not controlled autonomously by germ cells, but rather, by closely associated somatic cells of the gonad. Through genetic analyses, I find that somatic FGF ligand, Pyramus, and germline FGFR, Heartless, are required for maintenance of RC F-actin. Depletion of either is sufficient to deplete RC F-actin, which compromises stability of the RC and allows for cyst abscission – a defect that is rarely observed in wild type conditions. Within the germline, we link FGFR activation to a Src64 - Arp2/3 pathway known to regulate RC F-actin. Importantly, in both the fly and mammalian gonads, defects in somatic differentiation are known to induce formation of germ cell tumors (GCTs). By temporally controlling induction of soma-derived germ cell tumors, our live imaging has revealed that loss of F-actin at RCs and inappropriate cyst abscission are the first cellular defects evident in GCTs. Additionally, we find that tumor induction coincides with loss of FGF activity in the germline. In all, our work identifies a novel somatic signal required to control germline incomplete cytokinesis and implicated in disease.

128 **Novel roles for nuclear pore components in sperm development** Danielle B Buglak, Carey J Fagerstrom, Nasser M Rusan National Heart, Lung, and Blood Institute

During spermiogenesis, spermatids undergo dramatic morphological changes to develop into mature sperm. The final architecture of the sperm requires stable attachment of the axoneme (sperm tail) to the needle shaped nucleus (sperm head). Secure attachment is mediated by the head-tail coupling apparatus (HTCA) and its failure results in male sterility due to sperm decapitation. The molecular components required for secure linkage at the HTCA are not well understood, but recent evidence suggests that nuclear pore complexes (NPC) are important for male fertility and sperm development. Here, we investigated the role of the NPC in *Drosophila* sperm development at the HTCA using a testis-specific RNAi screen of individual NPC components. After knockdown, we assessed spermatids for various HTCA phenotypes, including sperm decapitation and abnormal connections between the head and tail. We found that Nup133, a component of the NPC outer rings, is required for establishing the initial attachment between the sperm head and tail. Loss of Nup133 led to sperm decapitation early in development, along with mislocalization of key HTCA proteins and abnormal nuclear shaping. Nup133 localized to the developing manchette, a microtubule-based structure important for nuclear shaping and HTCA establishment. We hypothesize that Nup133 is necessary for dynein anchorage at the nuclear envelope, which is necessary to bring the sperm head and tail together to form an initial attachment. Interestingly, we found that Nup93-2, a component of the NPC inner ring, is not required for initial attachment at the HTCA, but functions later in spermiogenesis to ensure proper alignment between the head and tail. This suggests that the NPC may serve a secondary function in HTCA maintenance. We hypothesize that Nup93-2 may stabilize and anchor the microtubule manchette to keep the HTCA properly aligned. Our work suggests that the NPC may serve multiple functions across different stages of spermiogenesis. We are currently working to determine the specific molecular functions of Nup133 and Nup93-2 in HTCA establishment and maintenance.

129 **Genetic and cellular processes regulating sperm length: Insights from *D. pseudoobscura* testis single cell RNAseq** Fiona Messer[1], April Talbot[2], Sabrina Williams[2], Helen White-Cooper[2] [1]Biosciences, Cardiff University, [2]Cardiff University

Sperm length is genetically controlled, and consistent within a single male and a single species, even in the face of environmental variability. Sperm differentiation is thus an excellent model system for investigation of intrinsic cell size control mechanisms. *Drosophila pseudoobscura* (*D. pse*) males produce sperm of three distinct lengths. Each sperm morph grows very reproducibly to a specific length, strongly suggesting that this process is under precise genetic control. Thus, *D. pse* spermatogenesis provides an ideal system to study the molecular mechanisms controlling cell size (sperm length).

D. pse sperm production is continuous; all stages are present within adult testes. Germline and somatic stem cells reside at the apical region of the testis. Encapsulation of a spermatogonium by two cyst cells generates a cyst; division and then differentiation of germline cells occurs synchronously within each cyst. Each cyst produces either long (300µm), medium (100µm) or short (50µm) sperm. Only long sperm fertilise the egg. The mechanisms underpinning production of distinct sperm lengths remain unexplored.

To address this question, we used single cell RNAseq of whole *D. pse* testes. Clustering identified a single population of germline stem cells and spermatogonia, suggesting that these early cells are not yet committed to a specific morph. The first evidence of differential transcriptomes was apparent in late spermatogonia – a split generated two major trajectories which remained separated for the remainder of spermiogenesis. Cross validation with FISH identified that this transcriptome split corresponds with the differentiation of long vs medium/short sperm. A second split occurs in spermatocytes on the medium/short branch.

Significant differential gene expression was evident between morphologically indistinguishable cells. 'Long' spermatocytes were more transcriptionally active (total counts and total genes), than medium/short precursors. 'Long' spermatocytes showed higher expression of genes involved in axoneme assembly, cytoskeleton and cell membrane. An insight into transcriptional regulatory mechanisms is provided by differential expression of a suite of transcription factors early in spermatogenesis. Testis meiotic arrest complex (TMAC) components were upregulated in long and the TMAC regulator *kumgang* upregulated in medium/short. We are developing *D. pse* RNAi lines to experimentally examine the roles of these TFs in controlling sperm elongation.

130 **The Role of Ca^{2+} Signaling in Apoptosis-induced Proliferation** KOMAL SUTHAR, Andreas Bergmann Department of Molecular, Cell and Cancer Biology, UMass Chan Medical School

Calcium (Ca^{2+}) plays a major role in many cell biological processes to maintain tissue homeostasis. Apoptosis-induced Proliferation (AiP) is a process in which apoptotic cells send signals to surviving neighboring cells to induce their proliferation. To examine AiP, we are using the "undead" AiP model in larval eye imaginal discs where simultaneous overexpression of the proapoptotic gene *hid* and the effector caspase inhibitor *p35* uncouples the functions of the initiator caspase Dronc for apoptosis and AiP. In the undead model, Dronc activates the NADPH oxidase DUOX to generate extracellular reactive oxygen species (ROS) which is a required step for AiP. The mechanism by which Dronc activates DUOX is unknown. DUOX contains two EF-hand motifs which are known to bind Ca^{2+}. Here, we identified Ca^{2+} entry into the cytosol as a significant factor for DUOX activation during AiP. Importantly, Ca^{2+} entry is dependent on the caspase Dronc in an apoptosis-independent manner. Three cell surface Ca^{2+} channels from the TRP family (TRPA1, TRPM and PKD2) mediate Ca^{2+} influx. Additionally, calcium-induced calcium release (CICR) from the ER mediated by the Ryanodine Receptor serves as another source of cytosolic Ca^{2+} during AiP, and accounts for Ca^{2+} oscillations. We are currently examining the exact role of the EF-hands of DUOX in AiP. In summary, these findings demonstrate the importance of Ca^{2+} signaling for DUOX activation during AiP and further underscore the dual role of caspases in apoptosis as well as in promoting cell proliferation and tissue regeneration under specific conditions.

131 **A novel micropeptide modulates *Drosophila* development, metabolism and stress response** Shyama Nandakumar, Deepika Vasudevan Cell Biology, University of Pittsburgh

The eukaryotic transcription factor ATF4 regulates the Integrated Stress Response, which responds to various stressors such as ER stress and nutrient deprivation. When ISR is activated, global protein synthesis is swiftly dampened due to the phospho-inactivation of the initiator methionine complex by stress-responsive kinases. However, ATF4 translation is paradoxically induced due to its specialized 5' leader which contains multiple short upstream ORFs (uORFs). Our work uncovers that the ultimate uORF (uORF2) preceding the ATF4 main ORF encodes for a functional micropeptide we nicknamed μP^{ATF4}. Under homeostasis, the ribosome initiates translation at uORF1 and occasionally reinitiates translation at the uORF2 start codon to synthesize μP^{ATF4}. However, under conditions of stress, reinitiation at μP^{ATF4} decreases due to reduced initiator methionine, thus allowing the ribosome to reinitiate at the ATF4 start codon. Since the sequence of uORF2 partially overlaps with that of ATF4 but is out of frame, this mechanism implies a trade-off between synthesis of μP^{ATF4} and ATF4, such that μP^{ATF4} is favored under homeostasis and ATF4 under stress. Accordingly, we detect robust μP^{ATF4} expression in many tissues where *ATF4* mRNA is present but ATF4 activity is not detected. However, overexpression of μP^{ATF4} in tissues with high basal levels of ATF4 protein such as the larval fat body resulted in suppression of ATF4 activity. Consistent with this observation, μP^{ATF4} overexpression also suppressed ATF4 induction in response to stress in human cells indicating that μP^{ATF4}-mediated ATF4 regulation is an evolutionarily conserved mechanism.

Fat-body specific overexpression of μP^{ATF4} results in precociously wandering larvae, which intriguingly also display a prolonged wandering phenotype. These animals also show higher lipid reserves, suggesting that μP^{ATF4} plays important roles in physiology and metabolism as well. We are currently investigating whether these phenotypes are ATF4-dependent given the known roles for ATF4 in lipid metabolism. Our preliminary data show that μP^{ATF4} acts on the 5' leader of ATF4, suggesting that μP^{ATF4} may be a regulator of mRNA translation. Consequently, we are testing this paradigm of regulatory logic on other stress response genes which encode similar 5' mRNA leaders. Together, our work uncovers a key modality of stress-mediated translation regulation and demonstrates a role for this mechanism in development and lipid metabolism.

132 **Erebosis is a heme-dependent non-apoptotic cell death in Drosophila enterocytes** Motohiro Morikawa, Sa Kan Yoo Laboratory for Homeodynamics, RIKEN BDR

Cell death and proliferation maintain tissue homeostasis. In Drosophila gut enterocytes, we recently discovered a novel form of cell death, named erebosis. Erebosis is characterized as a non-apoptotic, non-autophagic, and non-necrotic process. Erebotic cells accumulate Ance (angiotensin converting enzyme) and lose virtually all other proteins and organelles. The underlying mechanism of erebosis has yet to be elucidated.

Here, through single-cell RNA-seq and genetic screening, we found the small metabolite heme controls erebosis. Cells undergoing erebosis upregulated the heme degrading enzyme *Heme oxygenase* (*Ho*) and the heme exporter *Mrp5*. Heme depletion by *Mrp5* overexpression promoted erebosis while heme accumulation by knockdown of *Ho* or *Mrp5* inhibited erebosis. Suppression of erebosis reduced intestinal stem cell proliferation, indicating a cross-talk mechanism between enterocyte erebosis and stem cell division. The elevation of cytoplasmic heme in enterocytes did not induce ROS or apoptosis, phenomena typically associated with heme excess. Our results indicate that the reduction of cytoplasmic heme is a critical step in initiating enterocyte erebosis and stem cell division, thus maintaining the gut tissue homeostasis. This work provides the first insight into the regulatory mechanism of erebosis.

133 **Isoform-specific functions of the phosphatidylserine sensor Orion reveal unknown eat-me signals in engulfment and degeneration of neurites** Nicolas G Vergara Ruiz, Bei Wang, Xinchen Chen, Yifan Shen, Chun Han Weill Institute for Cell and Molecular Biology and Department of Molecular Biology and Genetics, Cornell University

A hallmark of a healthy nervous system is the effective clearance of unnecessary or damaged neuronal branches by resident phagocytes. Phagocytic clearance of neuronal branches is triggered by "eat-me" signals externalized on the surface of neurons. The lipid phosphatidylserine (PS) is a universal neuronal "eat-me" signal, which is recognized by a chemokine-like extracellular PS sensor called Orion in Drosophila. Although we have demonstrated that Orion binds both exposed PS and the engulfment receptor Draper to facilitate phagocytosis, how Orion carries out this bridging function remains poorly understood. In addition, Orion has two protein isoforms with a shared C-terminal domain and distinct N-terminal domains. Whether the two isoforms possess distinct activities is unknown. To address these questions, we identified and mutagenized residues potentially mediating PS and Drpr interactions in each isoform based on protein structure predictions. The mutant Orion variants were then evaluated in vivo using multiple dendrite degeneration assays in Drosophila class IV dendritic arborization neurons. Our results show that both the PS- and Drpr-interacting interfaces reside in the shared C-terminal domain. Interestingly, however, we find that Orion(A) is significantly more potent than the Orion(B) isoform in inducing dendrite degeneration and engulfment, indicating an N-terminal domain-mediated and Drpr-independent destruction function of Orion(A). Lastly, genetic interaction analyses of Orion and Eato, an ABCA lipid transporter that is required for preventing engulfment of dendrites by phagocytes, suggest that Orion(A) recognizes an unknown eat-me signal exposed by Eato mutant neurons while Orion(B) cannot. Together, these findings provide important mechanistic insights into the recognition of PS by endogenous sensors, and reveal novel layers of regulation of neuronal engulfment by PS sensor interactions and eat-me signals.

134 **The role of p53 and Dronc in regeneration following necrosis** Jordan Hieronymus, Robin Harris Arizona State University

Diverse types of injuries from trauma, infection and disease can lead to necrotic tissue death. Necrosis is a form of lytic cell death that can occur in every multicellular organism, resulting in complex and poorly understood tissue outcomes. Due to the uncontrolled and variable nature of necrosis, understanding this phenomenon is challenging. Our lab has leveraged the regenerative potential of the Drosophila wing imaginal disc to explore necrosis using a genetic manipulation system called DUAL Control. This system allows us to induce necrosis in the distal wing pouch and genetically manipulate the surrounding tissue.

We found that necrotic cell death results in caspase activity, a phenomenon normally indicative of apoptosis, at a distance from the injury. We called these cells “Necrosis-induced caspase-positive cells” (NiCP). However, NiCP do not behave like normal apoptotic cells, as they fail to undergo apoptotic signaling and do not die. Importantly, NiCP appear to be essential for successful regeneration. What causes NiCP and how they impact regeneration remains unclear.

To understand what mediates NiCP formation and function, we investigated different elements known to be involved in causing caspase activity. We found both the apoptotic regulator p53 and the initiator caspase Dronc are required. p53 is a well-studied transcription factor, known to be upregulated following tissue damage. Interfering with p53 specifically in the region where NiCP occurs using DUAL Control results in a significant decrease in NiCP cells and loss of regeneration after necrosis. Additionally, altering Dronc activity by expressing a catalytically inactive form also reduces NiCP and the regenerative response. As Dronc is known to be involved in processes other than apoptosis, such as tissue homeostasis, proliferation, and development, these findings suggest that non-apoptotic caspase activity is important for regeneration following necrosis, while p53 potentially regulates this process. To further understand the complexities of these pathways in NiCP formation and regeneration we are using whole genome sequencing targeting NiCP cells to provide a comprehensive overview of the important genetic changes involved. Ultimately, we hope to further our understanding of the role of non-apoptotic caspases in recovery from tissue necrosis.

135 **Tumour cell death and its fundamental role in establishing a pro-tumourigenic microenvironment** Jane Lyon, Andrew J Davidson School of Cancer Sciences, University of Glasgow

Drosophila has played a historically important role in understanding both the mechanism of programmed cell death and its consequences for the living tissue that is left behind. The Davidson lab utilises novel, genetically-encoded biosensors of apoptotic cell death to live-image developmental cell death and its clearance (Raymond*, Davidson*, *et al*., Science 2022). Furthermore, we are also able to induce and label necrotic tissue damage during acute injury, including through three-colour, live-imaging (Davidson *et al*., Nat Cell Biol. 2024). We are applying these unique tools to comprehensively visualising tumour cell death and investigating its paradoxical role in establishing a pro-tumourigenic microenvironment. Leveraging the decades of seminal work arising from the study of tumourigenesis in the wing disc, we have established a pupal wing model wherein we can spatially and temporally control tumour initiation. The optic tractability of the pupa, combined with unpublished variants of our apoptotic biosensors, allows the live-imaging of the growing and dying parts of the tumour in real-time. Furthermore, independent labelling of the phagocytic plasmatocytes (hemocytes/macrophages) using the QF system allows the dynamic interaction between these immune cells and the transformed tissue to be captured.

Within this model, we have quantified tumour cell death in the classic rasv12/dlg RNAi model of tumourigenesis. Furthermore, tracking of the plasmatocytes has revealed their recruitment into the nascent tumour microenvironment and their clearance of tumour apoptosis therein. Utilising *Drosophila’s* unrivalled genetics, we have suppressed tumour apoptosis within the transformed tissue and studied the consequences for tumourigenesis and plasmatocyte recruitment. Inhibition of apoptosis is unlikely to suppress all tumour cell death and our ability to live-image tumour necrosis is allowing us to explore the effect of different types of cell death on tumourigenesis, with important consequences for how we go about trying to treat cancer in the clinic.

Here, we will present our novel tools, model and live-imaging and demonstrate how we are using them to interrogate the tangled relationship between tumourigenesis, innate immune cells and cell death.

136 **Endogenous OptoRhoGEFs reveal biophysical principles of epithelial tissue furrowing** Andrew D Countryman[1], Caroline A Doherty[2,3], Marisol Herrera-Perez[4], Stanislav Y Shvartsman[2,3], Karen E Kasza[5] [1]Biomedical Engineering, Columbia University, [2]Molecular Biology, Princeton University, [3]Lewis-Sigler Institute for Integrative Genomics, Princeton University, [4]Biomedical Engineering, University of Rochester, [5]Mechanical Engineering, Columbia University

During development, epithelia function as malleable materials that undergo extensive remodeling to shape developing embryos. Optogenetic control of Rho signaling provides an avenue to investigate the mechanisms of epithelial morphogenesis, but transgenic optogenetic tools can be limited by variability in tool expression levels and deleterious effects of transgenic overexpression on development. Here, we use CRISPR/Cas9 to tag *Drosophila* RhoGEF2 and Cysts/Dp114RhoGEF with components of the iLID/SspB optogenetic heterodimer, producing healthy, homozygous-viable flies with consistent tool expression levels and permitting light-dependent control over endogenous protein activities. We develop an approach to precisely manipulate Rho activity levels at a subcellular scale by quantitatively controlling iLID photoconversion. Using these quantitative optogenetic perturbations, we uncover a dose-dependence of tissue furrow depth and bending behavior on RhoGEF recruitment, revealing mechanisms by which developing embryos can shape tissues into particular morphologies. We show that at the onset of gastrulation, furrows formed by cell lateral contraction are oriented and size-constrained by a stiff basal actomyosin layer. This sheds light on strategies employed by the ventral and cephalic furrows to permit tissue internalization within the mechanical context of the primary epithelium at this developmental stage and clarifies why lateral contraction is employed in the formation of small, narrow furrows in the primary epithelium. Furthermore, we demonstrate the applicability of the endogenous OptoRhoGEFs in mechanically manipulating epithelial tissues across a range of developmental processes, including cellularization and dorsal closure. Our findings demonstrate the use of quantitative, 3D-patterned perturbations of cell contractility to precisely shape tissue structures and interrogate developmental mechanics.

137 **Gene network divergence gives rise to three distinct organs during embryogenesis from homologous primordia.** Daniel Barcenilla-Merino, Carlos Sánchez-Higueras, James C-G Hombría Centro Andaluz de Biología del Desarrollo (CABD) (CSIC-UPO-JA)

Our laboratory has previously shown that, despite their distinct functions and shape, the endocrine organs controlling moulting and metamorphosis and the tracheal system, originate from homologous metameric organs.

During embryogenesis, the tracheal network develops from ten metameric ectodermal primordia expressing *ventral veinless* (*vvl)* which are specified from the second thoracic to the eighth abdominal segments, while the endocrine organs derive from homologous cells in cephalic segments also expressing *vvl*. Congruent with their homologous character, glands and tracheae can be homeotically transformed into each other, and *vvl* expression is regulated through the same cis-regulatory element, activated by a similar upstream gene network involving *Hox*, *Hedgehog, Wingless*, and *JAK/STAT* pathways. The difference between these organs is established by the expression of the transcription factor Trachealess in the tracheal primordia, while the gland primordia activate the Epithelial-to Mesenchymal Transition (EMT) inducer Snail (Sna), which promotes the loss of epithelial characteristics and induces their collective cell migration.

Here we show that a third organ, the tentorium, a conserved cephalic endoskeletal organ in insects, also derives from the same metameric *vvl* expressing cells. The tentorium is specified in the head from primordia adjacent to those forming the glands, and it resembles the trachea by not expressing Sna and maintaining epithelial characteristics during migration.

Studying tentorium organogenesis is challenging due to the segment fusions and reorganization occurring during head involution. To overcome these challenges, we have generated reporter genes allowing us tracking tentorium morphogenesis *in vivo* from its specification at stage 11, through invagination and reorganization up to its final integration with other elements that form the cephalopharyngeal head skeleton.

I will present experiments using *in vivo* and fixed embryos to elucidate the complex development of this conserved apodeme during *Drosophila* embryogenesis and describe the gene networks responsible for the divergence of these homologous metameric organs.

138 **Chitin is a critical determinant for generating curvature in the *Drosophila* corneal lens** Neha Ghosh, Jessica E. Treisman Department of Cell Biology, NYU School of Medicine

The *Drosophila* corneal lens is a biconvex structure of which a major component is the complex polysaccharide chitin. Our recent work shows that in *dusky-like* mutants, chitin deposition is delayed, and the adult corneal lens surface is flat and rough. However, it is not known whether chitin is essential to generate the precise curvature of the corneal lens. The chitin synthase protein encoded by *krotzkopf verkehrt* (*kkv*) is expressed by all the non-neuronal cone and pigment cells. Knocking down *kkv* in all these cells causes loss of chitin and makes the corneal lenses thinner. Conversely, over-expression of the chitin deposition gene *rebuf* (*reb*) in all retinal cells results in expanded and deformed corneal lenses. These data suggest that the normal level and distribution of chitin are essential for corneal lens shape development. The cone and primary pigment cells, collectively called central cells, lie below the thick central region of the corneal lens, while the secondary and tertiary pigment cells (lattice cells) are attached to the tapered corneal lens edges. We found that *kkv* knockdown only in the cone and primary pigment cells produced adult corneal lenses which were flatter and contained minimal chitin. Thus, we identified the central cells as the major source of corneal lens chitin. Consistent with this, we find that chitin synthesis in the retina begins at the mid-pupal stage, with radial chitin microfibrils first appearing over the primary pigment cells and later expanding to the cone cells. By late pupal stages the chitin fibers are organized into regular layers which are maintained in the adult corneal lenses. We tested the effect of localized increases in chitin by overexpressing *reb* in central cells or lattice cells. We observed that over-production of chitin specifically from the central cells gave the corneal lenses a spherical shape, while producing excess chitin from lattice cells expanded the corneal lens edges, producing more rectangular corneal lenses. These data indicate that chitin distribution is critical for determining corneal lens architecture.

139 **Comparative Analysis of Homologous Gene Functions using *Drosophila melanogaster*** Makenna Dunkel, Megan McCabe, Ava Negahdar, Kunie Yoshinaga-Sakurai, Dongyu Jia Kennesaw State University

Many cancers are initiated by the activation of oncogenes, which promote a cancer phenotype through a variety of disruptive mechanisms, including excessive proliferation, loss of cell differentiation, and inappropriate cell migration. The mechanisms can be summarized in an organizational system known as the hallmarks of cancer. *FYN* is a human oncogene implicated in many cancers, especially those of the central nervous system and ovary. *FYN* encodes a non-receptor tyrosine kinase from the *Src* tyrosine kinase family, and has been shown in literature to promote proliferation and metastasis. Data from Jia Lab previously demonstrated that human *FYN* expression in the *Drosophila* eye resulted in a deleterious effect on eye structure, suggesting potential conserved functions across species. This project utilized the *Gal4-UAS* system to express *FYN* and its closest *Drosophila* homolog, *Src64B*, in the eye and ovary of *Drosophila melanogaster*, to further explore and characterize the mechanisms of *FYN*. Our findings suggested that *FYN* and *Src64B* exhibit conserved phenotypes when expressed in the eye and ovary. *FYN* and *Src64B* expression in the eye demonstrated a conserved rough eye phenotype, including disruption of ommatidia organization and lost or shortened bristles, indicating developmental defects. *FYN* expression in the *Drosophila* ovary demonstrated accumulation of anterior egg chamber squamous cells without associated proliferative activity. These accumulated squamous cells exhibited altered expressions of apical, basolateral, and *Par*-complex polarity factors. Overexpression of Src64B showed similar phenotypes. These results indicate that the conserved oncogenic potential of *FYN* may be linked to changes in cell polarity signaling, which is considered a hallmark of cancer.

140 **How to divide a multinucleated cell – lessons from myofiber splitting of Drosophila indirect flight muscle** Shiv Sharma[1], Paula A Hernandez[2,3], Elizabeth Chen[4,5,6,7] [1]Department of Molecular Biology, University of Texas Southwestern Medical Center, Dallas, [2]Department of Orthopaedic Surgery, University of Texas Southwestern Medical Center, Dallas, [3]Department of Biomedical Engineering, University of Texas Southwestern Medical Center,Dallas, [4]Department of Molecular Biology, University of Texas Southwestern Medical Center, Dallas, TX, USA, [5]Department of Cell Biology University of Texas Southwestern Medical Center, Dallas, TX, USA, [6]Hamon Center for Regenerative Science and Medicine University of Texas Southwestern Medical Center, Dallas, TX, USA, [7]Harold C. Simmons Comprehensive Cancer Center, University of Texas Southwestern Medical Center, Dallas, TX, USA.

While most cell-cell fusion events generate multinucleated cells, most cell division events begin with a mononucleated parent cell that divides into mononucleated daughter cells. It has been a longstanding question as to how a multinucleated cell divides into two daughter cells. Here, we use the splitting of Drosophila indirect flight muscle (IFM) as a model to address this question. During pupal muscle development in metamorphosis, each multinucleated IFM divides into two multinucleated myofibers. Our genetic, and live imaging analyses revealed several unexpected cellular events leading to IFM division. First, muscle precursor cells (MPCs) infiltrate the mechanically soft, multinucleated myofiber to form a cell-in-myofiber structure. The infiltration process is facilitated by DE-cadherin-mediated adhesion between MPCs and the myofiber. The infiltrated MPCs then upregulate Notch expression and rapidly proliferate within the myofiber, leading to a significant increase in the number of muscle cells aligned along the midline of the myofiber. The increased number of mononucleated muscle cells exert mechanical forces to push the existing myonuclei to opposite edges of the myofiber. Gradually, the plasma membrane of the myofiber is pulled apart around the midline by the mechanical forces, leading to the splitting of the myofiber into two multinucleated daughter fibers. During the splitting process, the muscle cells within the myofiber continue to differentiate and fuse with the inner myofiber membrane, thus adding nuclei to the myofiber and contribute to its growth. Taken together, our study has revealed, for the first time, a novel mechanism of how a multinucleated cell is divided into two daughter cells and highlighted critical functions of cell infiltration, proliferation, and mechanical forces in this process.

141 **Adult *Drosophila* salivary gland cells exhibit alternative polarity and mode of cell division** Gary R Hime[1], Caitlin van Ree[2], Harshaa Chandrasekaran[2], Nicole Dominado[2], Nicole A Siddall[2], Izaac L Moran[2] [1]Anatomy and Physiology, University of Melbourne, [2]University of Melbourne

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, little is known of the development of adult *Drosophila* salivary glands except that they consist of a single layer, tubular epithelium that originates from a population of diploid cells found as an imaginal ring near larval salivary gland ducts. We have shown that the adult salivary glands contain three distinct epithelial domains, two of which are comprised of cuboidal epithelial cells and one of squamous epithelial cells. These cell types develop during the pupal period and after eclosion secretory cells develop extensive apical membrane invaginations. The junctional polarity of the epithelial cells exhibits an unusual change soon after eclosion as adherens junctions migrate from a position apical to the septate junction to a more basal position. The polarity factors aPKC, Baz, Crb, Dlg1 and Scrib, as well as members of the integrin adhesion complex protein localise to discrete sub-cellular domains within cuboidal epithelial cells. Knockdown of *scrib* within these cells results in a loss of apicobasal polarity.

Adult salivary gland epithelial cells are polyploid and increase in number immediately post-eclosion yet do not undergo mitosis. By using genetic tools designed for the MARCM lineage tracing technique we have shown that the polyploid cells lose chromosomes during the division period and appear to be using amitosis as a mechanism to increase cell number. We have identified the first evidence for amitosis involvement in primary formation of a tissue and the adult *Drosophila* salivary gland will serve as a model for genetic analysis of this mode of division, and for study of junctional reorganisation within epithelia.

142 **Hippo's Dynamic Duo": How Yorkie and Wingless Orchestrate Tumor Growth?** Arushi Rai[1], Amit Singh[1,2,3,4], Madhuri Kango-Singh[1,2,3,4] [1]BIOLOGY, University of Dayton, [2]Center for Tissue Regeneration & Engineering (TREND), University of Dayton, [3]Premedical Programs, University of Dayton, [4]Integrative Science and Engineering (ISE), University of Dayton

Human cancers such as pancreatic and lung adenocarcinomas with oncogenic KRAS mutations and disruptions in cell polarity regulators (e.g., Scribble) demonstrate significant modulation of evolutionarily conserved pathways, including Wingless (Wg), Hippo, MAPK, and apoptosis. Using well-established *Drosophila* $Ras^{V12},scrib^{-/-}$ tumor models, we found a dynamic regulatory network including Wg which acts upstream of this network, regulating caspases, JNK, and Yki that regulates tumor growth. In this study, we hypothesize that Yorkie (Yki)is the key downstream regulator of this network. Wg is ectopically induced in Ras^{V12} and $Ras^{V12},scrib^{-/-}$ clones. We have further observed that downregulating Wg signaling ($dTCF^{DN}$; $Ras^{V12},scrib^{-/-}$) significantly reduces tumor clone size, suppresses survival signaling (low DIAP1 expression), and decreases apoptotic activity. Similarly, reducing *yki* dosage (heterozygosity *of yki*) in $yki^{B5}/+$; $Ras^{V12},scrib^{-/-}$ clones moderates clone size, alters apoptotic response, and decreases survival signaling. To further investigate the synergistic effects, we generated double mutant MARCM clones ($dTCF^{DN}$ $yki^{B5-/-}$; $Ras^{V12},scrib^{-/-}$) to simultaneously suppress Wg and Yki activities. Using these mosaic tumor models, we devised experiments to study the following aspects of tumorigenesis: a) Cancer Hallmarks: Invasion, cell adhesion, and survival signaling assessed by immunohistochemistry; b) Transcriptional Networks: mRNA expression studies via qRT-PCR; and c) Pathway Activities: Western blot analysis of Hippo, JNK, and Wg signaling. Preliminary results reveal that compared to $Ras^{V12},scrib^{-/-}$ model, suppression of Wg and Yki together leads to significant changes in tumor growth dynamics and suppresses aggressive tumor growth. These findings provide novel insights into how Yki and Wg cooperatively regulate tumorigenesis in $Ras^{V12},scrib^{-/-}$ tumor model. Our data provide mechanistic insights about Yki/YAP-mediated tumor growth, specifically the key nodes and molecular interactions between these conserved pathways, which may have significance for devising treatment approaches for KRAS-driven malignancies.

Keywords: Tumorigenesis, Yorkie, Wingless, Hippo pathway, $Ras^{V12},scrib^{-/-}$ *Drosophila*, Cancer Hallmarks, Molecular Networks

143 **Peptide hormone shapes lipid-steroid metabolic states to trigger sexual maturation** Jie Sun[1], Hong-Cun Bao[2], Adam Aldahir[2], Wen-Kan Liu[3], Calder Ellsworth[2], Yi-Chun Huang[2], Wu-Min Deng[2] [1]Biochemistry and Molecular Biology, Tulane University, [2]Tulane University, [3]University of Illinois Urbana-Champaign

Steroid hormones are ancient and conserved signaling molecules that serve as crucial regulators in sexual development. The storage of steroid hormones in steroidogenic tissues is very limited, so for timely secretion, lipid droplet (LD) within endocrine cells must respond rapidly to stimuli, releasing free cholesterol (FC) as a precursor for steroid hormone synthesis. Here, we show that a brain-derived peptide hormone signal, the fly leptin analog *unpaired1* (*Upd1*), plays a pivotal role in precisely and efficiently maintaining the balance of LD pool and sterol pool in the steroidogenic prothoracic gland (PG). The spatiotemporal expression of Upd during development activates JAK/STAT signaling in the PG to precisely control developmental transitions, a regulatory effect that dramatically amplified in cases of tissue damage or tumorigenesis. The fluctuations in JAK/STAT signaling rapidly reprogram the metabolic state within the PG, precisely and robustly regulating the conversion between LD-cholesteryl ester (CE)- FC-steroid axes to fine-tune systemic steroid pulse frequency, amplitude, and duration. Unexpectedly, the sex determination factor Fruitless (Fru) functions as a downstream effector of JAK/STAT signaling, mediating sex-hormone synthesis through its own dynamic expression. We further identified the evolutionarily conserved hormone-sensitive lipase (Hsl), a major neutral cholesterol esterase mediating the conversion of CEs to FC, as the key rate-limiting enzyme responsible for the homeostasis of the Fru-guided LD-CE-FC-steroid axis. The deletion of JAK/STAT signaling pathway or Fru in PG cells disrupts the homeostasis of LD pool and sterol pool, leading to premature sexual maturation, a condition that can be significantly alleviated by the introduction of exogenous Hsl. Correspondingly, high levels of JAK/STAT signaling delay developmental transitions by inducing Fru/Hsl expression, and this mechanism is conserved in processes where injury, inflammation, and cancer interfere with development. In addition, we introduced steroidogenic tissue models from German cockroaches and mice, and found that the conserved JAK/STAT pathway couples with Hsl to regulate the delicate balance of LD-CE-FC-steroids, guiding the decision-making process for steroidogenesis. Our results demonstrate that a conserved regulatory network in the sexual development of both insects and vertebrates triggers sexual maturation by remodeling the LD-CE-FC-steroid metabolic states.

144 **PKA/RAF/ERK signaling interactions in brain learning and memory circuitry** James Sears[1], Kendal Broadie[2] [1]Biological Sciences, Vanderbilt Brain Institute, Vanderbilt University, [2]Biological Sciences, Vanderbilt Brain Institute, Cell and Developmental Biology, Pharmacology, The Vanderbilt Kennedy Center, Vanderbilt University and Medical Center

Both Protein Kinase A (PKA) and Rapidly Accelerated Fibrosarcoma (Raf)/Extracellular Signal-Regulated Kinase (ERK) pathways are critical for learning and memory plasticity, but their signaling interactions are not at all well understood. PKA and ERK pathways are also perturbed in seizure states, prompting investigation of disease-relevant interactions. PKA can phosphorylate Raf to both promote and prevent ERK function in context-specific mechanisms, but there is wide disagreement about contributions of the highly conserved phosphorylation residues. Here, we use PKA/ERK pathway genetic manipulations paired with PKA-/ERK-specific separation of phases-based activity reporter of kinase (SPARK) biosensors to test interactions in the *Drosophila* brain Mushroom Body (MB) learning and memory center Kenyon cell (KC) neurons. KCs are divided into 3 classes with distinctive learning/memory functions: α/β, α'/β', and γ. RhoGEF Trio labeling separates Trio+ α'/β' and γ from Trio- α/β KCs. We previously discovered a surprising phenotype when Raf^{gof}, a constitutively-active Raf, promoted ERK-SPARK signaling only in the Trio+ KC neurons. Since this effect is inversely correlated with the Trio- baseline PKA-SPARK signaling, we hypothesized ERK is limited by PKA. Consistently, we find that 1) baseline ERK signaling is elevated by PKA inhibition in Trio- and Trio+ KCs, 2) Raf^{gof} promotes PKA signaling in Trio+ KCs, and 3) ERK signaling with Raf^{gof} is strikingly extended and stronger in all KCs when PKA is inhibited. We conclude that there is a feedback inhibition loop through which Raf activity promotes PKA activity, which in turn restricts ERK signaling. We therefore suggest that PKA acts as a critical brake on ERK signaling to optimize learning/memory signaling, potentially through a mechanism in which PKA phosphorylates Raf. Together, our results suggest the hypothesis that activity-dependent Raf/ERK signaling is limited by feedback PKA inhibition in learning/memory circuitry. If so, these findings can be applied to seizure models to determine beneficial vs. harmful adaptations in low-threshold brain activity networks. This work is supported by NIH R01 NS131557.

145 **Toll-7 acts through Fra/DCC-dependent and -independent pathways to guide commissural axons across the midline** Sarah Gagnon[1], Yixin Zang[2], Greg J Bashaw[3] [1]University of Pennsylvania, [2]Zuckerman Institute, Columbia University, [3]Neuroscience, University of Pennsylvania

Commissural neurons send their axons across the midline to connect to contralateral targets. This is essential for left-right coordination, and defects in this process are associated with several neurodevelopmental disorders. In flies, the guidance receptor Frazzled (Fra), like its human ortholog Deleted in Colorectal Cancer (DCC), signals through a canonical pathway to induce cytoskeletal changes that drive growth toward the midline in response to its ligand Netrin. Fra also acts independently of Netrin through a non-canonical pathway to activate expression of *commissureless*, which in turn degrades the repulsive receptor Roundabout. Fra therefore promotes midline crossing through Netrin-dependent attraction and Netrin-independent inhibition of repulsion. Elucidating the mechanisms of Fra canonical and non-canonical pathways is essential to understanding how distinct signaling events are coordinated to achieve precise neural circuit wiring. We performed a mass spectrometry screen in fly embryonic neurons and identified the toll-like receptor family member Toll-7 as a novel Fra interactor. Previous work has shown that Toll-7 promotes motor and olfactory axon targeting, but its earlier developmental functions and potential role in midline circuit formation are unknown. Here we present evidence that Toll-7 acts both with Fra and independently of Fra to promote axon growth across the midline. We show that *toll-7* is expressed in the central nervous system at the time of midline crossing, consistent with a role in commissural axon guidance. Notably, we find that *toll-7* mutant embryos display axon guidance defects that partly overlap with those of *fra* mutants, including midline crossing defects and breaks in longitudinal axon tracks. Transgenic expression of *toll-7* in commissural neurons rescues the crossing defects, indicating a cell-autonomous function for Toll-7 in promoting commissural axon guidance. Furthermore, loss of a single copy of *toll-7* enhances the midline defects of hypomorphic mutants with attenuated Fra signaling, suggesting that Toll-7 acts with Fra to promote midline crossing. Interestingly, *fra, toll-7* double mutants show more severe midline defects than *fra* single mutants, suggesting that Toll-7 plays additional, Fra-independent roles at the midline. In addition, we find that Fra and Toll-7 interact through their cytodomains, and that the conserved Fra P motifs and Toll-7 TIR domain are dispensable for this interaction. Future experiments will determine which Fra pathway Toll-7 acts through to promote midline crossing, and whether canonical Toll/NF-κB signaling underlies the Fra-independent function of Toll-7. Altogether, our work uncovers a new function for Toll-7 in the assembly of neural circuits and sheds light on uncharacterized mechanisms of Fra signaling.

146 **Retinal Calcium Waves Regulate Tissue Patterning of the Fly Eye.** Ben Jiwon Choi, Yen-chung Chen, Claude Desplan Biology, New York University

Precise cellular arrangement is essential for optimal information processing in the nervous system. We show that synchronized calcium activity plays a critical role in patterning non-neuronal cells, shaping tissue architecture in the eye. We discovered robust calcium waves among non-neuronal retinal supporting cells during early eye development. These waves follow stereotyped initiation and propagation patterns, and do not involve photoreceptor neurons. They are driven by the receptor tyrosine kinase Cad96Ca-PLCγ-IP_3R pathway, triggering calcium release from the ER. The waves propagate through gap junctions, with specific combinations of innexins playing cell-type-specific roles. Retinal waves proportionally scale calcium signals to match ommatidial size, activating the Myosin II pathway to drive interommatidial cell contraction. This process compensates for regional size differences, shaping uniform boundaries that ensure consistent lens architecture for optimal visual function.

Our findings highlight how synchronized calcium activity shapes tissue architecture, offering insights into fundamental mechanisms that underlie nervous system development and function.

147 **Cell-surface proteomic profiling of the trachea-wing disc interface identifies proteins required for cytoneme formation and morphogen signaling** Wanpeng Wang, Thomas Kornberg CVRI, UCSF Morphogens dictate organ development and tissue patterning in multicellular organisms, but the mechanisms that disperse morphogens across tissues has been controversial. Evidence now suggests that morphogens are dispersed by specialized filopodia called cytonemes. Cytoneme-mediated signaling has been implicated in the *Drosophila* wing epithelium, testes, hematopoietic stem cell niche, heart and in vertebrate embryos. Using the trachea-wing disc system, we have shown that cytoneme-mediated signaling is synaptic, and neuronal synapse components, as well as Ca^{2+} influx, are essential for cytoneme mediated signaling. These findings challenge our understanding of morphogen gradient formation and suggest a deep homology between contact-based cell-cell communication in neurons and non-neuronal cells.

To understand the cytoneme-mediated morphogen signaling interface at the systems level, we carried out a trachea cell surface proteomic profiling study. HRP that is targeted to the trachea cell membrane combined with a short exposure to H_2O_2 and a membrane impermeable biotin substrate were used to label proteins at the trachea-wing disc interface. Quantitative profiling of the resulting biotin-enriched proteome revealed proteins required for neuronal axon guidance and wiring, extracellular matrix function and mechano-sensing. A proteome-instructed *in vivo* screen identified novel factors that are required for cytoneme mediated morphogen signalin including PlexB, Ten-m and Piezo1. We also discovered VEGF signaling between the trachea and wing disc. Combining spatial proteomics and genetic analyses, this study advanced our understanding of the cytoneme mediated morphogen signaling interface.

148 **Uncovering circadian mechanisms underpinning anti-aging benefits of time-restricted feeding (TRF)** Timothy Chang[1,2], Jared Gatto[3], Meaghan Jankowski[4], Andrés Martínez-Muñiz[3], Adriana Vélez-Alicea[5], Jennifer Hurley[4], Julie Canman[6], Mimi Shirasu-Hiza[3] [1]Department of Biological Sciences, Columbia University, [2]Biological Sciences, Columbia University, [3]Department of Genetics and Development, Columbia University Irving Medical Center, [4]Department of Biological Sciences, Rensselaer Polytechnic Institute, [5]Columbia Doctoral Program in Neurobiology and Behavior, Columbia University Irving Medical Center, [6]Department of Pathology and Cell Biology, Columbia University Irving Medical Center

Almost all animals have ~24-hour oscillations in biological functions known as circadian rhythms. These rhythms are set by molecular pacemakers known as the circadian clock, which exists in nearly every tissue tested. Proper circadian regulation is crucial for alignment of behavior and physiology with the environment; most animals (including humans) exhibit loss or weakening of circadian regulation with aging and disease. Loss of circadian regulation is associated with increased pathology; yet few effective treatments currently exist to treat chronic loss of circadian rhythms. In recent years, time-restricted feeding (TRF) has emerged as a promising approach to ameliorate circadian dysregulation, promote health, and extend lifespan. Because TRF entails restricting food intake to specific hours of the day rather than restricting caloric and nutrient content, TRF has been hypothesized to depend on circadian-regulated functions including many metabolic pathways. Previously, 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. We showed that iTRF enhanced transcriptional oscillations in circadian clock and autophagy components, both of which were required for iTRF-mediated lifespan extension. Because the precise mechanisms driving TRF-induced benefits for aging remain largely unclear, we conducted an RNA-sequencing experiment at both 10 and 30 days after iTRF versus an *ad libitum* diet. Early analyses show that enhanced oscillation of core circadian components was induced by iTRF after only 10 days of treatment. We also saw an overall decrease in gene oscillations with age that is partially ameliorated by iTRF. We are currently identifying and characterizing genes that constitute candidates for driving anti-aging iTRF-mediated health benefits, particularly those that lost or gained circadian oscillation due to iTRF, and will test these candidates genetically for their role in aging and lifespan.

149 **Determining the role of dVGLUT in sex specific differences in Parkinson's Disease models.** Kevin Garzillo, Daniel Babcock Biological Sciences, Lehigh University

Parkinson's Disease (PD) is the fastest growing neurodegenerative disease worldwide. It is characterized by bradykinesia, tremor, rigidity, and the accumulation of Lewy Bodies in the substantia nigra pars compacta (SNpc). The motor symptoms of PD arise as a result of the progressive loss of dopamine (DA) neurons in the SNpc. PD disproportionately affects males, as they are twice as likely to develop PD, tend to develop symptoms earlier, experience more severe motor impairments, and exhibit greater neurodegeneration than females. Despite these clear sexes differences, the mechanism by which female physiology protects against PD is unknown. *Drosophila* is an ideal model for studying sex differences in PD due to its quick generation time, genetic tractability, cell-autonomous sex determination, and conservation of many PD related genes. We found that expressing wild type or mutant α-synuclein, the main component of Lewy bodies, in *Drosophila* DA neurons recapitulates the sex differences observed in human PD. Male flies developed motor symptoms earlier, exhibited greater motor impairment, and exhibited more severe neurodegeneration than females. These differences in motor impairment and DA neuron degeneration were not due to differences in transgene expression or α-synuclein protein abundance. Selective masculinization of female DA neurons via knockdown of the sex determination gene Transformer (Tra) eliminated the observed differences in climbing ability by increasing the severity of motor deficits in females. Previous studies have demonstrated that expression of Vesicular Glutamate Transporter (VGLUT) is lower in male than in female DA neurons. VGLUT expression has been linked to neuronal resilience in PD, as VGLUT-positive cells in the SNpc were demonstrated to be resistant to degradation in mammalian PD models. Consistent with these results, we found that knockdown of VGLUT in females expressing mutant α-synuclein exacerbated motor dysfunction and neurodegeneration, abolishing the sex difference. Using the mitochondrial oxidative stress sensor, MitoTimer, we observed that oxidative stress is higher in male DA neurons than in females, suggesting that VGLUT may protect against PD pathology by reducing oxidative stress in female DA neurons. These results suggest that the mechanism of sex specific vulnerability in PD is due to differences in VGLUT expression.

150 ***Drosophila* models of GABA dysregulation in *SLC6A1*-neurodevelopmental disorder** Paige I Hall[1], Kristy L Jay[2], Jonathan C Andrews[1], Sharayu V Jangam[1], Ryan German[1], Vanessa A Gomez[1], Hongling Pan[1], Shinya Yamamoto[1], Michael F Wangler[1] [1]Molecular and Human Genetics, Baylor College of Medicine, [2]Center for Genomic Medicine, Harvard University

Drosophila have been used to model several rare diseases to elucidate mechanisms of pathogenesis. Baylor College of Medicine's Model Organism Screening Center brings new variants in both disease-associated and new genes to research environments, where we aim to uncover the role each variant has in causing disease. In humans, variants in the gene *SLC6A1* cause a rare neurodevelopmental disorder (*SLC6A1*-NDD) that presents with a broad phenotypic spectrum consisting of symptoms like epilepsy, autism spectrum disorder, intellectual disability, and more. Despite nearly 200 *SLC6A1*-NDD individuals being identified, the molecular mechanism of disease pathogenesis on a variant-by-variant basis remains unclear. In this study, we are leveraging the fly to better understand the disease mechanism of five variants: *SLC6A1*^{A288V}, *SLC6A1*^{S295L}, *SLC6A1*^{G297R}, *SLC6A1*^{F339L}, and *SLC6A1*^{A344S}. *SLC6A1* encodes the GABA transporter 1 (GAT1) protein that is responsible for GABA reuptake at the synapse in both neurons and glia. The *Drosophila* ortholog of *SLC6A1* is *Gat*, which encodes the single GABA transporter in the fly, Gat, and is exclusively expressed in glial cells. *Drosophila* make an excellent tool for modeling *SLC6A1*-NDD due to the similarity of GABA regulation between humans and the fly. Since GABA is the primary inhibitory neurotransmitter, impaired GABA reuptake due to impaired GABA transporters results in altered inhibitory signaling. Preliminary data suggest that our fly models recapitulate the phenotypes observed in patients, and each variant-expressing fly line shows varying levels of neurological and morphological changes. We have observed significant electroretinogram (ERG) defects suggestive of synaptic imbalances in the *SLC6A1*^{S295L} variant flies. Heat-shock based seizure assays have shown significantly increased seizure recovery times in several of the variant-expressing fly lines. Lastly, the variant-expressing flies have significantly decreased sleep latency and sleep consolidation compared to reference-expressing flies. Each of these phenotypes are a direct result of GABA dysregulation. In the future, we aim to rescue these phenotypes using an array of GABA-related therapeutics complemented by RNAi knockdown strategies to better understand cellular mechanisms causing disease-related phenotypes. Identifying therapeutics that best suppress these disease-related phenotypes by genotype will improve outcomes for those living with *SLC6A1*-NDD and other forms of epilepsy.

151 **Cortex glial subtypes in the central nervous system differentially regulate seizure susceptibility** Govind Kunduri, Usha Acharya, Jairaj Acharya Cancer and Developmental Biology Laboratory, National Cancer Institute

Glial cells permeate the mammalian nervous system. Conventionally glial cells have been studied as homogenous groups of cells that are structurally and functionally identical across brain regions. However, recent studies have shown that depending on the anatomical region in the brain, glia exhibit heterogeneity in their development, molecular profiles, and functions within glial subtypes. Existence of glial subtypes within the same class have also been identified in Drosophila, however, their functional significance remains unknown. In this study, we have taken a genetic approach to assess functional differences among cortical glial subtypes across brain regions. Towards this goal, we have developed transgenic tools to identify and manipulate cortex glial subpopulations in the larval, pupal and adult brain. We optimized cortex glial subtype specific expression pattern across brain regions in the optic lobe (OL), central brain (CB), and ventral nerve cord (VNC) using Gal4, splitGal4, Gal80, LexA and killer zipper systems. We have used these tools to explore the role of regional cortex glia in epilepsies. Previous studies have shown that Ceramide phosphoethanolamine synthase (*cpes*) mutants exhibit light inducible seizures while Na^+/Ca^{2+}, K^+ exchanger (*zyedeco/zyd*) mutants show temperature inducible seizures due to aberrant cortex glial function. Do cortex glia located in different parts of the brain regulate these seizure types differentially? To answer this question, we have performed distinct brain region specific rescue experiments in *cpes* and *zyd* mutants using the cortex glial subtype specific drivers developed in this study. We found that OL and CB, but not VNC specific cortex glial expression of UAS *CPES*, was able to significantly suppress light inducible seizures in *cpes* mutants. In contrast, VNC but not OL or CB specific cortex glial expression of UAS NCKX was able to suppress temperature sensitive seizures. Further, in a third model, expression and activation of transient receptor potential (TrpA1) just in the VNC specific cortex glia was sufficient to induce temperature sensitive seizures in wild type flies. Taken together our findings show that regionally specialized cortex glial subtypes differentially regulate seizure susceptibility, suggesting glial subtype heterogeneity has local functional implications.

152 ***Drosophila* models of genetic microcephaly identify conserved mechanisms of neurodevelopment.** Nicole Losurdo, Adriana Bibo, Nichole Link Neurobiology, University of Utah

We previously demonstrated that *Drosophila* is an excellent model to investigate human microcephaly, or reduced head and brain size. We identified primary microcephaly patients with variants in *ANKLE2*. Disruption of the fly ortholog also caused decreased brain volume in developing larvae. *Drosophila Ankle2* mutants were fully rescued by expression of wild type human *ANKLE2* but not with variants found in microcephaly patients. Our results showed that ANKLE2 is essential for neurodevelopment and is causative of human disease. Using ANKLE2 as a model, we assessed a large cohort of microcephaly patients with unsolved causes of disease to identify candidate genes required for brain development. Exome sequencing of patient and parents detected variants possibly linked to disease. A loss-of-function screening approach determined if conserved orthologs in *Drosophila* were required for brain development. *In vivo* RNAi was used to test the function of 45 candidate genes in neural stem cells and post-mitotic neurons. Using third-instar larval brain lobe volume as a proxy for brain size, we found 18 genes required in neural stem cells and 22 genes required in neurons for proper brain growth. Future work will investigate whether protein function is conserved between flies and humans and if so, whether variants found in microcephaly patients affect protein function. Lessons from our work may promote diagnoses for patients, identify novel genes associated with disease, and illuminate pathways essential for brain development.

153 **Dissecting the role of bulk lipid transporters in human disease: a novel movement disorder model** Sarah D Neuman[1], Rajan Thakur[2], Scott J. Gratz[2], Kate M. O'Connor-Giles[2], Arash Bashirullah[1] [1]Pharmaceutical Sciences Division, University of Wisconsin-Madison, [2]Department of Neuroscience, Brown University

Bridge-like lipid transfer proteins (BLTPs) mediate bulk lipid transport at membrane contact sites, with mutations linked to both early-onset neurodevelopmental and later-onset neurodegenerative diseases, including movement disorders. The tissue specificity and temporal requirements of BLTPs in disease pathogenesis, however, remain poorly understood. Although neuronal dysfunction is traditionally considered central to movement disorders, recent studies suggest that primary myopathy may also contribute, particularly in chorea-acanthocytosis (*VPS13A* disease). Using *Drosophila* models, we investigated the tissue- and temporal-specific roles of two BLTPs: *VPS13A* and *BLTP2*. We find that neuron-specific loss of the *VPS13A* ortholog, *Vps13*, causes age-onset movement deficits, neurodegeneration, and reduced lifespan. Notably, muscle-specific loss of *Vps13* affects only lifespan, revealing that neurodegeneration and myopathy are likely independent comorbidities in *VPS13A* disease. In contrast, neuronal loss of the *BLTP2* ortholog, *hobbit*, causes severe early-onset locomotor defects without signs of neurodegeneration, pointing to a developmental rather than maintenance role. Indeed, we find that *BLTP2/hobbit* functions postsynaptically to promote the formation of functional synapses. Our findings show that bulk lipid transport mediated by BLTPs is required both for formation of neuronal synapses and for maintenance of neuronal survival.

154 ***CNTN2* is a candidate modifier in a PIGA-CDG pedigree with reduced penetrance** Holly Thorpe[1], Brent Pedersen[2], Joshua Bonkowsky[2], Aaron Quinlan[1], Clement Chow[1] [1]Human Genetics, University of Utah, [2]University of Utah

Loss of function mutations in the X-linked *PIGA* gene lead to PIGA-CDG, an ultra-rare congenital disorder of glycosylation (CDG), typically presenting with seizures, hypotonia, and neurodevelopmental delay. We identified two brothers (probands) with PIGA-CDG, presenting with mild developmental delay, epilepsy, and autism. Both probands carry the novel, rare $PIGA^{S132C}$ variant, a predicted damaging variant not found in the gnomAD database. Confirming this diagnosis, both probands show a 50% decrease in GPI-anchor proteins on the cell surface. Strikingly, the maternal grandfather and a great uncle both also carry $PIGA^{S132C}$, but neither presents with symptoms associated with PIGA-CDG. We hypothesized that there might be a modifier segregating in the family that contributes to this reduced penetrance. Using whole genome sequencing and pedigree analysis, we identified all the possible susceptibility variants found in the probands and not in carriers and all the possible protective variants found in the carriers and not in the probands. This list of potential candidates included heterozygous, damaging variants in three other genes also involved directly in GPI-anchor biosynthesis, *PIGS, PGAP5,* and *DPM1*, and a small number of genes involved in other glycosylation pathways or encoding GPI-anchored proteins.

To functionally test our predicted modifiers, we used a *Drosophila* eye-based model of PIGA-CDG. We created double knockdowns (KD) of *PIGA* and the candidate modifiers and compared the eye sizes of the double KD to the PIGA eye model and single KD of the candidate modifiers. Multiple candidate modifiers showed significant interactions with *PIGA* in the fly that mimic what we predict in the family. *CNTN2*, a GPI-anchored protein harboring a nonsense variant, was the only predicted protective gene from the pedigree analyses to rescue the *PIGA* phenotype in *Drosophila*. Further testing of *CNTN2* was also performed in *Drosophila* neurological models of PIGA-CDG displaying seizures and climbing defects. Similar to the results seen in the eye model, loss of *CNTN2* rescued both seizures and climbing defects in the PIGA-CDG neurological models. This *Drosophila* functional validation makes *CNTN2* a strong candidate modifier underlying the incomplete penetrance seen in this family. However, it is unlikely it is the sole contributor. Identifying and studying rare disease modifier genes in human pedigrees may lead to pathways and targets that may be developed into therapies.

155 **Investigating the Synergy between the Gut Microbiome and Sweet Taste Receptors and their Impact on Glucose and Lipid Metabolism in Drosophila** Mikesha D. Carter Biology, San Francisco State University

Diabetes is the 7th leading cause of death globally and the most common metabolic disease. One of the major factors of diabetes is **obesity**, which can be caused by addictive behaviors towards food, especially foods high in sugar. It has been shown that individuals with diabetes have dulled sweet taste reception and develop addictive feeding behaviors towards sugar. The **gut microbiome** has also been associated with an increased risk of obesity and altered feeding behavior. The impact of sweet **gustatory receptor genes** and the gut microbiome influence on feeding preference, glucose and lipid metabolism is poorly understood. We are investigating these factors independently and in combination to observe their effects. These factors include Gr64a-f, a paralog to T1R2 and T1R3, and the gut microbiota can be altered in drosophila melanogaster to examine markers for glucose and lipid metabolism. As a model organism, Drosophila are easy to manipulate genetically and can be produced with conventional bacteria or as axenic (germ/bacteria-free) flies. Here we used a Binary Choice feeding assay and spectrophotometry to measure feeding preference. Also, confocal microscopy to measure the expression of Drosophila insulin-like peptide (DILP) and lipid droplet size which are markers for glucose metabolism and lipid metabolism, respectively.

156 **Mitochondrial function is a key regulator of adult fat body differentiation in development** Ignacio M Fernandez Guerrero, Beatriz Castejon Vega, Alberto Sanz School of Molecular Biosciences, University of Glasgow

Drosophila melanogaster is highly sensitive to mitochondrial dysfunction during development. In addition to drastically reduced lifespan, flies with impaired mitochondrial respiration from development displayed decreased starvation resistance and triglyceride levels, indicating absence of the adult fat body–confirmed by transcriptomics and microscopy. As a crucial organ for energy storage, detoxification, and immune response, the fat body is essential for maintaining metabolic and physiological homeostasis in *Drosophila*.

We induced mitochondrial dysfunction in the progenitor cells of the adult fat body by knocking down complex IV subunit COX5B. In pupal stages, these progenitor cells migrate from the thorax to the abdomen where they coalesce into four distinct bands before fusing and differentiating into adult fat body cells. Live imaging revealed that while COX5B-depleted progenitor cells follow normal migration patterns, they fail to differentiate. This fat body deficiency persists into adulthood, as evidenced by decreased starvation resistance and triglyceride levels in flies aged 5, 10, and 30 days. Interestingly, the lack of adult fat body has no negative effect on the lifespan of these flies. Differentiation of the adult fat body was rescued by expressing alternative oxidase (AOX), which partially restores mitochondrial function, in the COX5B-deficient progenitor cells. Altogether, this demonstrates that mitochondrial function is critical for successful differentiation of the adult fat body progenitor cells.

This research offers an insight into how mitochondria regulate cell fate decisions during differentiation, shedding light on the broader role of mitochondrial function in developmental biology.

157 **Bending the Rules: How Somatic Cell Mechanics Drives Reproductive Evolution** Suhrid Ghosh, Chandrashekar Kuyyamudi, Cassandra Extavour OEB/MCB, HHMI/Harvard University

During the development of the *Drosophila* ovary, somatic cells undergo complex morphogenetic processes that define the number of reproductive structures, known as ovarioles. Each ovariole forms through the precise stacking of terminal filament cells (TFCs), with a species-specific number of cells in each stack. The rules governing TFC stacking display striking evolutionary diversity: some fly species change the number of cells per stack, while others change stack numbers. However, the regulatory mechanisms behind these processes remain elusive.

To investigate the mechanisms that control cell numbers in TFC stacking, we began by characterizing terminal filament (TF) formation in *D. melanogaster*. First, we observed that TFC precursors are medio-laterally polarized and undergo rapid cytoskeletal rearrangements to form TF stack cells. Using *ex vivo* live imaging and theoretical modeling, we found that both the initial cell shape and cytoskeletal dynamics are essential for stack formation and maintenance.

Additionally, we discovered an anterior-to-posterior deformation gradient in TFC stacks that is conserved across species. Removing cortical tension flattens this gradient. Based on the assumption that new cells join the stack from the anterior, we hypothesize that the deformability of individual TFCs regulates the number of cells that can integrate into a stack. To test this, we modulated proteins involved in cortical stiffness in TFC precursors and found that altering cortical stiffness led to stable changes in TFC numbers per stack.

Finally, we developed a novel method to isolate single TFCs in vitro across fly species, enabling us to examine their mechanical properties. This approach allows us to trace the evolution of TFC mechanical properties at the single-cell level. Our findings highlight the role of cell mechanics within the gonadal soma as a determinant of reproductive fitness across species.

158 **Dpp and Defective Proventriculus: A Tug-of-War in Determining Eye and Head Fate** Anjali Sangeeth[1], Neha Gogia[2], Anuradha Chimata[3], Madhuri Kango-Singh[2,4,4,5,6], Amit Singh[2,2,4,5,6,7] [1]Department of Biology, University of Dayton, [2]Biology, University of Dayton, [3]University of Dayton, [4]Center for Tissue Regeneration and Engineering at Dayton (TREND), University of Dayton, [5]Premedical Program, University of Dayton, [6]Integrative Science and Engineering (ISE), University of Dayton, [7]Center for Genomic Advocacy (TCGA), Indiana State University

During organogenesis, the establishment of the Antero-Posterior (AP), Dorso-Ventral (DV), and Proximo-Distal (PD) axes is crucial for the transition from a two-dimensional organ primordium to a three-dimensional organ. These complex developmental processes rely on the coordinated actions of transcription factors, morphogens, and signaling pathways along spatio-temporal axes. Recently, we identified defective proventriculus (*dve*), a K-50 transcription factor, the ortholog of human SATB1, as a dorsal fate selector gene in eye development, which induces wingless (*wg*) to promote head-specific fate. In Drosophila eye, Decapentaplegic (Dpp), a member of the evolutionarily conserved Dpp/Bone Morphogenetic Protein (BMP) pathway, is crucial for initiating morphogenetic furrow progression at the posterior margin, regulating proliferation and retinal differentiation. Dpp interacts antagonistically with Wingless (Wg) to modulate retinal differentiation. In this study, we explore the interaction between the *dve* and the Dpp signaling pathway, highlighting how this interplay determines the fate of the Drosophila eye versus the head cuticle. Our findings indicate that Dpp and *dve* are mutually antagonistic in defining eye versus head identity. Misexpression of *dpp* in the head vertex region shifted the fate from head to eye, overriding the *wg* influence. This alteration was accompanied by negative regulation of Homothorax (hth) and ectopic expression of retinal differentiation factors. Moreover, we demonstrate that this interaction is conserved in mammals through SATB1, which has been implicated in hypertelorism, a craniofacial defect characterized by an abnormal increase in the distance between the eyes, resulting from disruptions in axial patterning processes.

159 **Effect of hemocyte migration patterns on extracellular matrix deposition and embryonic development** Elena Dapi, Marisol Herrera-Perez Biomedical Engineering, University of Rochester

During Drosophila embryonic development, hemocytes migrate throughout the embryo and deposit extracellular matrix (ECM) along the developing ventral nerve cord (VNC). Intrinsic stresses from the deposited ECM generate an autonomous viscous drag that contributes to the proper formation of the VNC. However, it is still unknown how specific patterns of hemocyte migration alter ECM distribution and thus the viscoelastic forces that help shape the VNC. To dissect this, we use a set of optogenetic tools to modulate RhoGEF2 localization and actomyosin contractility with high spatial and temporal precision in migrating hemocytes. We show that changes in RhoGEF2 localization result in altered myosin distribution that correlates with decreased cell locomotion and persistence, as well as an overall decrease in migration distance and speed compared to control embryos. Alterations in hemocyte migration patterns also result in reduced embryo viability (measured as hatching). We are evaluating the effects of altered hemocyte migration on VNC morphology and ECM deposition. We expect these studies to lay the groundwork for further analysis of the role of ECM pattern deformation and material viscosity properties as dynamic regulators of tissue formation in vivo.

160 **Spatial Resolution and Scaling Limitations of Tissue Extension Machinery in a Rapidly Remodeling Embryonic Epithelium** Liam J Russell[1], Rashmi Budhathoki[2], Todd Blankenship[2], Dinah Loerke[1] [1]Molecular and Cellular Biophysics, University of Denver, [2]Biological Sciences, University of Denver

During early development in the *Drosophila* embryo, robust mechanical and molecular programs coordinate to convergently extend the germband epithelium along the primary body axis. This process is driven by coordinated cell shape changes that cause rows of planar polarized cells to directionally intercalate with one another. However, these processes are dependent on informational systems that inform anterior-posterior identities and set up planar polarities in the embryonic epithelium. Here, we seek to examine the resolution of these patterning programs, and to determine how the dissemination of morphogenetic information is regulated spatially. To address this, we have examined embryos with mutations in the maternal haploid (*mh*) gene, which possess twice as many cells as wild-type embryos. Intriguingly, our preliminary results show significantly slowed rates of tissue extension in these small-celled embryos, both at the full tissue and individual intercalary motif levels. These reductions are larger than would be expected through the geometric considerations of half-sized cells alone. Further, we find that maternal haploid embryos have a smaller range of myosin II intensities, which could be explained by a breakdown of AP patterning information at this smaller scale. We also examined the impact of smaller cells on the oscillatory contractile behaviors that drive many morphogenetic processes. Here, the contractile machinery appears capable of oscillating half-sized cells with frequencies and amplitudes comparable to control, suggesting that oscillation machinery operates in a scale-independent fashion. Recent work from our lab has also highlighted the importance of nuclear size, shape, and positioning in permitting tissue remodeling processes. Individual haploid nuclei seem to be more tightly packed within their respective cells, but more loosely packed with respect to neighboring cells through increased apical-basal nuclear dispersion at the onset of tissue extension. While this increased dispersion would in theory result in a more fluid epithelium that could remodel more efficiently, our results indicate the contrary, suggesting that upregulated nuclear dispersion is not sufficient to overcome contractile defects in a crowded epithelium with increased cell densities.

161 **Understanding The Development of Key Somatic Cell Types in Drosophila Ovaries** Joanna Portillo[1], Abigail Dove[2], Mark Van Doren[2] [1]Biology, Johns Hopkins University, [2]Johns Hopkins University

Throughout the animal kingdom, male and female gonads develop differently in a process that is regulated by the Doublesex and mab-3 related (DMRT) family of transcription factors. Our lab studies how Drosophila Doublesex (Dsx) controls the development of the somatic gonad of the ovary and testis. Much is known about testis development, while much less is known about the development of the ovary. Therefore, to understand how Dsx controls sexual dimorphism in the somatic gonad, we first need to understand how the somatic cells of the ovary form and attain their distinct identities. The establishment of the somatic stem cells, Follicle Stem Cells (FSC), and somatic supporting cells, Escort Cells (EC), has not been well characterized. The current theory is that EC and FSC develop from a set of precursor somatic cells known as “intermingled cells” (ICs) in the larval ovary. Single-cell RNA sequencing of the larval ovary revealed that the ICs may have distinct anterior vs. posterior identities that may influence EC and FSC differentiation (Lehman Lab). In characterizing FSC development, our lab has found that a pool of FSC precursors forms from posterior ICs during early pupal stages. Additionally, we determined that the JAK/STAT pathway is required for cells to take on FSC precursor identity at this time. Based on these observations, our current work investigates autonomous and non-autonomous factors that may be involved in the establishment of EC and FSC identities, such as intrinsic IC identity and cell-cell signaling pathways. We have begun examining how the JAK/STAT and Hedgehog (HH) pathways influence EC and FSC development. Using the UAS/GAL4 system, we manipulated the level of autonomous signal the cells receive from these pathways. We then visualized cell identity and characteristic changes with confocal imagining through immunofluorescence and in-situ hybridization. Overexpression of JAK/STAT and HH results in the proliferation of ICs in larval gonads, which leads to a gross proliferation of EC and FSC in adult ovaries. The loss of JAK/STAT results in ovaries with no FSC and increased EC, while the loss of HH produces agametic ovaries. These results highlight the importance of both pathways in EC and FSC development. To further characterize the influence of these pathways on development, we are also determining if IC identity has been altered in a way that leads to changes in ECs and FSCs. In this way we can determine how different regional IC identities are established and how they lead to EC vs FSC development.

162 **Genetic tools for characterizing enteroendocrine cells in the *Drosophila* midgut** Ellen Popodi, Jessica Holsopple, Robert Eisman, Kevin Cook, Stephanie Mauthner Bloomington Drosophila Stock Center, Department of Biology, Indiana University

The *Drosophila melanogaster* midgut is a tractable model tissue for studying intestinal endocrine signaling. The intestine contains enteroendocrine cells, specialized epithelial cells that modulate gut physiology through hormone signaling. These secretory cells are highly diverse and produce distinct subsets of peptide hormones that regulate systemic responses to changing intestinal conditions. Genetic tools for identifying and experimentally manipulating these discrete cell types are essential to better understand the complex interactions between enteroendocrine cells and their hormone signaling pathways. Although the split-GAL4 system has been used to delineate some populations of enteroendocrine cells, their hormonal profiles and functional roles remain largely uncharacterized. Here, we investigate whether enteroendocrine subtypes, defined by pairs of split-GAL4 drivers, produce a limited set of peptide hormones. We surveyed the expression profile of peptide hormone genes in particular enteroendocrine cell types in the anterior, middle and posterior regions of the midgut using a set of lexA reporters that mimic the native expression of these genes. Our analysis reveals a rich array of peptide hormone expression within these cell subtypes. Furthermore, we observe unique peptide hormone expression patterns in discrete compartments of the midgut using split-GAL4 driver combinations that target smaller subsets of enteroendocrine cells. Our findings collectively highlight the complexity of enteroendocrine cell types and their hormonal repertoires, which may vary based on their regional location within the gut. Moreover, our work demonstrates the utility of split-GAL4 drivers for the precise identification and characterization of intestinal cell types. It also provides a simple framework for future studies investigating split-GAL4 driver combinations that target larval and adult tissues beyond the gut.

163 **A mitochondrial redox switch licenses the onset of morphogenesis in animals** Updip Kahlon[1], Francesco Dalla Ricca[1], Saraswathi Pillai[1], Marine Olivetta[2], Kevin Tharp[3], Li-En Jao[4], Omaya Dudin[2], Kent McDonald[5], Mustafa Aydogan[1] [1]University of California, San Francisco, [2]University of Geneva, [3]Sanford Burnham Prebys Medical Discovery Institute, [4]UC Davis, [5]UC Berkeley

Embryos undergo pre-gastrulation cleavage cycles to generate a critical cell mass before transitioning to morphogenesis. The molecular underpinnings of this transition have traditionally centered on genetic cues from nuclei, as repressing zygotic chromatin remodeling and genome activation can prevent downstream processes of differentiation and organogenesis. Whether chemical cues from the cytoplasm could play a role in this transition, however, remains unknown. Despite precedents that oxygen depletion can similarly suspend development in early embryos, hinting at a pivotal role for oxygen metabolism, whether there is a *bona fide* chemical switch that licenses the onset of morphogenesis is unclear. Here we discover that a mitochondrial oxidant acts as a metabolic switch to license the onset of animal morphogenesis. Concomitant with mitochondrial activation, we found a burst-like accumulation of mitochondrial superoxide (O_2^-) during *Drosophila* blastoderm formation. *In vivo* chemistry experiments revealed that an electron leak from site III_{Qo} at ETC Complex III is responsible for O_2^- production. Importantly, depleting mitochondrial O_2^- fully mimics anoxic conditions and, like anoxia, induces suspended animation prior to morphogenesis, but not after. Specifically, H_2O_2, and not $ONOO^-$, NO, or HO•, can single-handedly account for this mtROS-based response. We demonstrate that depleting mitochondrial O_2^- similarly prevents the onset of morphogenetic events in vertebrate embryos and ichthyosporea, close relatives of animals. We postulate that such redox-based metabolic licensing of morphogenesis is an ancient trait of holozoans that couples the availability of oxygen to development, conserved from early-diverging animal relatives to vertebrates.

164 **Melanization regulates wound healing by limiting polyploid cell growth in the *Drosophila* abdominal epithelium** Loiselle Gonzalez[1], Elizabeth Mortati[2], Lillie Mitchell[2], Vicki Losick[2] [1]Biology, Boston College, [2]Boston College

Wound healing requires a localized response that restricts growth, remodeling, and inflammation to the site of injury. In the fruit fly, *Drosophila melanogaster*, the epithelium heals a puncture wound through cell growth, instead of cell division. Epithelial cells on wound margin both fuse and duplicate their genome to generate a multinucleated, polyploid cell essential for tissue repair. Polyploid cells contain more than the diploid copy of the genome and in the adult fly epithelium arise by the endocycle and cell fusion in response to injury. Despite the essential role of polyploidy in wound healing, the signals that initiate and regulate the extent of cell growth at the wound site remain poorly understood. The first step in wound healing following a puncture injury requires generation of melanin scab to repair the Drosophila cuticle. The melanized scab forms at the wound site within hours and is dependent on the activation of the pro-phenoloxidases (PPO1, PPO2, and PPO3). Using a triple PPO null mutant, we have uncovered a novel role for melanization in regulating wound healing by limiting polyploid cell growth after injury. Thus, we have found that melanization is required for efficient wound closure, yet the loss of melanization leads to unexpected exacerbation of polyploid cell growth in surrounding epithelial cells. This occurs in part through the early entry of epithelial cells into the endocycle which may be due to altered gene expression as a result of delayed JNK signaling and other pathways. In conclusion, we have found that polyploid cell growth requires melanization at the injury site to control the extent of cell growth post injury and instruct efficient wound closure.

165 ***Lactobacillus brevis* induces regenerative response in *Drosophila* through ornithine** Gloria Bates[1], Christian Dimayuga[2], Hadi Ammar[3,4], Aki Ohdera[1], Zevin Condiotte[1], Jesus del Rio Salgado[1], Yutian Li[1], Joshua Thaosatien[5], Lea Goentoro[6] [1]Biology & Bioengineering, California Institute of Technology, [2]Division of Natural Sciences, Pasadena City College, [3]Polytechnic School, [4]University of California, Berkeley, [5]Stanford University, [6]California Institute of Technology

In this study, we examined the impact of modifying the gut microbiome on the host regenerative responses. As an injury model, we used the adult *Drosophila* limb, which does not normally regenerate upon injury. We find that feeding the flies *Lactobacillus brevis* induces regenerative responses in the amputated limb. Regenerative responses are defined by modulation of wound healing, enhanced tissue survival, and, eventually, partial regrowth of the limb. To investigate the microbial contributions, we performed genome-scale metabolic modeling of *L. brevis* and measured microbial secretions in the fly. We find that the specific *L. brevis* strain used in this study secretes the amino acid L-ornithine. Ornithine in the context of promoting tissue regrowth is interesting because ornithine is a key intermediate in upcycling nitrogen. Therefore, we further investigated the impact of ornithine. First, we confirmed that flies fed *L. brevis* show higher ornithine levels and show modulation in the levels of enzymes that metabolize ornithine. Then, we tested and found that directly feeding ornithine recapitulates the regenerative responses induced by *L brevis*. These experiments supports a model in which modifying the gut microbiome, specifically by enriching *L. brevis*, can induce regenerative responses, and identifies a metabolite involved in the host-microbe interactions that promotes regeneration.

166 **Nrf2/CncC and Hsf1 play a role in intestinal stem cell identity and gut homeostasis in *Drosophila*** Imilce A Rodriguez-Fernandez[1], Carlos Quiñones Sánchez[1], Airined Montes[1], Heinrich Jasper[2] [1]Biology, University of Puerto Rico Rio Piedras, [2]Genentech

As organisms age, tissue renewal and repair after injury declines. Adult somatic stem cells play a key role in preserving tissue regeneration, and they do this in part by their unique capacity to withstand cellular stress throughout the organism's lifespan. Understanding the molecular mechanisms that sustain stem cell function could pave the way for developing therapies to combat age-associated tissue degeneration and promote healthspan (the disease-free period of life).

We are interested in how adult somatic stem cells can endure cellular stressors such as oxidative and proteostatic stress. Using D*rosophila melanogaster* intestinal stem cells (ISCs) as a model system, we previously found that ISCs from young flies employ the transcription factor Nrf2/CncC - master regulator of the antioxidant response- to maintain protein homeostasis. We also found that reactivating Nrf2/CncC in old ISCs promoted protein homeostasis, resulting in lifespan extension and healthspan.

For this study, we explored the role of Nrf2/CncC and Hsf1, two conserved stress-sensing transcription factors, in regulating stem cell function and found an unprecedented role of these genes in ISC identity and the regulation of the gut microbiome. We generated young flies in which each individual transcription factor or both simultaneously were knocked down in ISCs. Then, their guts were dissected, their ISC sorted, and RNA sequencing was performed. RNAseq of ISCs revealed that Nrf2/CncC and HSF1 individually repress different genes linked to proliferation, and, unexpectedly, both transcription factors repress the EE-specification gene *asense*. Knockdown of both transcription factors led to an accumulation of EE progenitors and the early onset of aging-like phenotypes, including microbial dysbiosis, leaky gut, and reduced survival. To characterize the gut bacteria changes at the species level we performed full-length 16s rRNA sequencing (~1500 bp) using PacBio technologies. Shannon index analysis for alpha diversity and Bray-Curtis Dissimilarity index for beta diversity showed an increase in microbial diversity when both transcription factors are knocked down in the ISCs. These findings reveal that Nrf2/CncC and Hsf1 play crucial roles in regulating stem cell identity in ISCs, and that compromised expression of these factors leads to microbial dysbiosis. This sheds light on age-related mechanisms contributing to stem cell misdifferentiation and dysbiosis.

167 **The *Drosophila* testis compensates for catastrophic germ cell loss by altering stem cell cytokinesis** Christie Campbell[1], Tiffany Roach[2], Kari F Lenhart[2] [1]Biology, Drexel University, [2]Drexel University

Tissue homeostasis requires a balance in production of self-renewing stem cells and differentiating daughters, or progenitors. The specialized microenvironment, or niche, in which stem cells reside provide regulatory signals to control this balance. Diminished niche signals and subsequent progenitor loss, or atrophy, is a hallmark of aging. It has been found that in many systems, stem cells respond to progenitor loss by inducing increased rates of stem cell divisions. However, the specific mechanisms by which stem cells and their progenitors respond to tissue atrophy remain elusive. Using the *Drosophila* male germline as a model system, we explored a genetic model that depletes progenitors to mimic tissue atrophy through ablation by expression of an apoptotic factor, Hid. During homeostasis, germline stem cells (GSCs) engage a delayed cytokinesis program, the timing of which is controlled by niche derived Jak/STAT signaling. Following contractile ring disassembly, GSCs pause cytokinesis progress through formation of a secondary F-actin ring; the ring's persistence is controlled by Jak/STAT signaling and determines the timing of cytokinesis completion and release of daughter cells. Previous work from our lab has shown that fast cycling GSCs consistently fail to complete cytokinesis, and thus, mitotic rate may be deleterious as a compensatory mechanism. Here, we elucidate a novel mechanism used by the testis niche to compensate for tissue atrophy. Ablation of germ cells leads to compensatory feedback to the niche, not only inducing faster GSC cycling, but *also* faster disassembly of the secondary actin ring, thus significantly shortening the cytokinetic pause. Together, these changes in GSC behavior promote substantially faster release of progenitors from the niche. This altered cytokinesis timing is achieved through increased Jak/STAT signaling in the niche, mediated by increased niche area. We are currently investigating the hypothesis that differentiating somatic cells move back to the niche and transdifferentiate to increase niche size. Together, this data supports a model whereby under catastrophic germ cell loss, a larger niche is formed, providing increased STAT signal to support a faster release of progenitor cells. Future work will investigate the mechanistic steps underlying this feedback and subsequent change in niche architecture to better understand how stem cell niches attempt to prevent tissue atrophy.

168 **CG15312 suppresses apoptosis in long-lived *Drosophila* hindgut enterocytes** Jessica K Sawyer[1], Ruth A Montague[2], Paulo Belato[2], Olivia Goddard[2], Don Fox[2] [1]Duke University, [2]Department of Pharmacology & Cancer Biology, Duke University

Maintaining tissue homeostasis in the presence of injurious signals requires diverse molecular mechanisms. Many studies focus on the role of cell replacement through regeneration in tissue maintenance. However, as an alternate to regeneration, many tissues contain long-lived cells. How such cells resist injury and are maintained is poorly understood. Here, we show that the adult *Drosophila* hindgut ileum is a model to uncover mechanisms of tissue longevity and injury resistance. First, we find that the *Drosophila* hindgut ileal enterocytes last the lifetime of the organism and are resistant to cellular insults. Ileal enterocytes resist cell death via SDS feeding and to pro-apoptotic signals in the *H99* locus, *hid* and *rpr*. In contrast, midgut enterocytes are sensitive to both of these insults. This apoptotic insensitivity is specific to *H99*/*hid*, as ectopic expression of a downstream *hid* target, caspase *dronc* (Caspase-9), kills adult ileal enterocytes. Unlike the compensatory hypertrophic regenerative response to cell death in the neighboring hindgut pylorus after *hid* expression as we have previously described, *dronc*-mediated cell death in the ileum does not activate a robust regenerative response, highlighting the importance of cell maintenance in this intestinal epithelium. The *hid*-sensitive pylorus and *hid*-insensitive ileum are derived from the same progenitor population during metamorphosis. *hid*-sensitivity arises during development, as *hid* expression causes injury and cell death in the ileum in newly eclosed young adult animals. Our results suggest that, as an alternate to regeneration, long-lived, non-regenerative intestinal enterocytes of the ileum activate resistance to cellular insults as the adult hindgut matures. To understand the mechanisms of resistance, we isolated ileums from young and mature adults and performed RNAseq. Then, we conducted an RNAi screen of genes with elevated RNA levels in mature ileums. One of our top hits is a poorly characterized Immunoglobulin family cell adhesion gene, *CG15312*. Loss of this gene in a *hid* overexpression background leads to disruption of epithelial integrity and cell death. Further, loss of *CG15312* alone leads to a reduction in *FasIII* accumulation in ileal cell-cell borders. Our results suggest a novel molecular link between adhesion, cell death resistance, and tissue longevity.

169 **Transformation of enteroendocrine cell identity by the stress-inducible transcription factor *Xrp1*** Qingyin Qian[1,1], Makoto Hayashi[1], Hiroki Nagai[2], Yuya Sanaki[1], Ken-ichi Kimura[1], Satoru Kobayashi[1], Yuichiro Nakajima[2], Ryusuke Niwa[1] [1]University of Tsukuba, [2]The University of Tokyo

Cell plasticity describes the ability of a cell to change its identity. It is commonly depicted by progenitor's differentiation into progeny, which is fundamental to development. Furthermore, this typical top-down hierarchy can be reversed or traversed via de-differentiation or trans-differentiation, which is implicated in regeneration and diseases, or via forced knockdown/overexpression of critical genes.

The mechanism underlying such potency of a cell to alter its identity can be investigated using intestinal epithelial cells (IECs) of the fruit fly *Drosophila melanogaster.* Within IECs of adult *Drosophila*, intestinal stem cells (ISCs) give rise to committed progenitor cells, enteroendocrine progenitor cells (EEPs), and enteroblasts (EBs), thereafter respectively differentiating into secretory enteroendocrine cells (EEs) and absorptive enterocytes (ECs). Given EEs' importance in nutrient sensation and hormone production, we aim to characterize EE plasticity, upon external perturbation or with experimental manipulation.

With lineage tracing, we observed that a small number of EEs could change their identity into either ISCs or ECs, raising possibilities of de-differentiaion or trans-differentiation. We hence conducted single-cell RNA sequencing (scRNA-seq) of EEs and EE-derived cells, among which we annotated several EE clusters, one ISC/EB cluster, and two EC clusters, EC1 and EC2. Despite both EC1 and EC2 being positive for EC marker, EC2 expressed EB marker as well, while EC1 didn't. Genes differentially expressed in EC2 over EC1 were related to differentiation and regeneration. EC2 could thus be designated as an intermediate cell state for differentiation.

We then knocked down or overexpressed genes highly expressed in EC2 to examine whether EC2 was associated with EE identity changes. As a result, we narrowed our focus down to *Xrp1*, a stress-responsive transcription factor. We found that its EE-specific overexpression (O/E) induced EEs to change their identity, in a manner dependent on JNK signaling Cells derived from *Xrp1* O/E EEs had a nuclear size comparable to that of polyploid ECs and could express either EB or EC marker. This observation concorded with scRNA-seq, where EC2-enriched *Xrp1* bridged between EEs and EE-derived ECs.

Taken together, our results so far have provided molecular evidence of EEs' plasticity and proposed a stress-responsive EE fate conversion model. Ongoing research aims to identify any external perturbation that elicits *Xrp1* expression in EEs and to clarify the effect of *Xrp1* at a single-cell level.

170 **Membrane-bending proteins promote formation of a curved apical domain during *de novo* polarization in intestinal stem cell progeny** Anthony Galenza[1], Vibhu Guru[1], Elsa Su[1], Paola Moreno-Roman[1], Irina Kolotuev[2], Lucy Erin O'Brien[1] [1]Stanford University School of Medicine, [2]Université de Lausanne

Barrier epithelial tissues require apical-basal polarity and continual cellular regeneration to maintain their protective function. In the adult *Drosophila* intestine, basally located stem cell progeny–known as enteroblasts–are initially unpolarized and must establish their apical domain as they integrate into the mature barrier tissue. We (Galenza 2023) and others (Chen 2022) found that enteroblasts form their microvilli-lined apical membrane by first building a bulbous, asymmetric intercellular lumen, the Pre-assembled Apical Compartment (PAC). Using Focused Ion Beam-Scanning Electron Microscopy, we observed that PACs exhibit profound membrane curvature, bending nearly 180˚. This suggests that PAC formation depends on specialized membrane-bending proteins such as BAR (Bin-Amphiphysin-Rvs) domain-containing proteins. Mining the Fly Cell Atlas (Li 2022) for BAR-domain proteins enriched in differentiating enteroblasts, we identified 16 candidates. A systematic RNAi screen led us to focus on two candidates, Endophilin A (EndoA) and Nervous Wreck (Nwk), both historically associated with synaptic vesicle endocytosis. *endoA* and *nwk* are required for enteroblast differentiation and PAC formation. EndoA localizes to the cytoplasm specifically in differentiating enteroblasts, whereas Nwk is enriched at the apical membrane in both differentiating cells and mature enterocytes. Our work identifies EndoA and Nwk as prime candidates for PAC formation and implies that stage-specific deployment of BAR-domain proteins shapes the dynamic morphogenesis of bulbous PACs to enable integration of new cells into the intestinal barrier epithelium.

171 **BubR1 and Mad2 regulate adult midgut remodeling in *Drosophila* diapause** Yuya Adachi[1], Hiroki Nagai[1,2], Masayuki Miura[1], Yuichiro Nakajima[1] [1]Graduate School of Pharmaceutical Sciences, The University of Tokyo, [2]Institute of Science and Technology Austria

Diapause is a survival strategy in which growth and aging are temporarily suspended, enabling animals to withstand unfavorable environments. Various insects, including the fruit fly *Drosophila*, enter reproductive arrest, or dormancy, in response to cold temperatures and/or short-day lengths. During reproductive diapause, ovarian development halts, and non-reproductive organs also undergo remodeling at both morphological and metabolic levels; however, the mechanisms underlying this remodeling and its physiological impact remain largely unclear.

Here, we show that the *Drosophila* adult midgut undergoes extensive remodeling in diapause, marked by a sustained growth arrest due to the cell cycle arrest of intestinal stem cells (ISCs), reverting to normal upon returning to recovery conditions. Such adult midgut remodeling occurs in the model organism *D. melanogaster* as well as other *Drosophila* species such as *D. bifasciata* and *D. triauraria*, both of which naturally enter reproductive diapause in winter. Mechanistically, BubR1 and Mad2, key regulators of mitosis and components of the mitotic checkpoint complex, are highly expressed and localized in the cytoplasm of ISCs during dormancy, rather than at the kinetochore, and both BubR1 and Mad2 are essential for diapause-specific midgut remodeling. Furthermore, disruption of midgut growth arrest during diapause reduces the resistance to starvation in adult flies. Therefore, our findings identify a novel role for BubR1 and Mad2 in ISCs, promoting proper midgut remodeling during dormancy, and highlight the importance of this process for survival under adverse environments.

172 **State-of-the-art CRISPR screening technologies for *Drosophila* research and beyond** Stephanie Mohr Harvard Medical School

The *Drosophila* RNAi Screening Center (DRSC) was founded in 2004 to support genome-wide RNAi screens in *Drosophila* cultured cells by the community. Since then, we have expanded the suite of technologies and resources we make available to the community, including development of platforms for CRISPR knockout, knockdown, and over-expression screening. I will present an overview of the technologies and resources we have developed, then discuss how we continue to innovate through development of new technology platforms, resources, and assays. I will also touch on how we collaborate with others to use CRISPR cell screening to study diverse topics related to human health, including through extension of the CRISPR cell screen platforms to arthropod vectors of disease.

173 **The Multiomics Aging and Prolongevity Fly Cell Atlas (MAP-FCA): a Resource for Unraveling the Dynamics of Gene Regulation during Aging<nordpass-listeners></nordpass-listeners>** Tyler Jackson[1,2,3], Bo Sun[1,2], Yanyan Qi[1,2], Ye-Jin Park[1,2,4], Tzu-Chiao Lu[1,2], Christina Ko[5], Hongjie Li[1,2] [1]Molecular and Human Genetics, Baylor College of Medicine, [2]Huffington Center on Aging, Baylor College of Medicine, [3]Cancer and Cell Biology, Baylor College of Medicine, [4]Development, Disease Models, and Therapeutics, Baylor College of Medicine, [5]Neuroscience, Rice University

Understanding complex biological processes at the organismal level requires powerful tools that can simultaneously capture multiple layers of molecular information. To address this gap, we have developed a whole-organism single-nucleus multiome ATAC+RNA sequencing (sn-multiomics) pipeline for *Drosophila*. This cutting-edge platform enables the simultaneous measurement of chromatin accessibility and gene expression at single-cell resolution, offering unprecedented insight into gene regulatory networks across diverse tissues. Using this technology, we generated the Multiomics Aging and Pro-longevity Fly Cell Atlas (MAP-FCA), profiling more than 200 cell types with over 500,000 nuclei from normal aging and pro-longevity conditions across multiple ages. This dataset integrates both RNA and ATAC data to provide precise cell-type annotations and detailed chromatin landscapes. By leveraging the MAP-FCA, we performed integrative analyses to identify critical regulatory changes at the level of chromatin accessibility and transcriptional dynamics, providing new insights into cellular processes during aging. The MAP-FCA not only offers a comprehensive molecular map of *Drosophila* biology but also demonstrates the power of sn-multiomics in understanding the complex process of aging. In addition, our new sn-multiomics pipeline will serve as a valuable resource for the broader scientific community, enabling more accurate molecular profiling and offering a blueprint for future multiomics applications in model organisms.<nordpass-listeners></nordpass-listeners>

174 **Driving a protective single allelic variant of the mosquito FREP1 gene to combat malaria** Zhiqian Li, Lang You, Lei Yang, Ethan Bier University of California

Fibrinogen-Related Protein 1 (FREP1) is a mosquito host factor required for malarial parasites to traverse the midgut epithelium. The naturally-occurring FREP1Q allele is reported to prevent parasite infection, while supporting essential physiological functions in the mosquito. We generate congenic single-nucleotide edited strains of Anopheles stephensi, carrying either the putative parasite susceptible FREP1L224 or refractory FREP1Q224 alleles. The FREP1Q224 allele confers robust resistance to infection by both human and rodent malarial parasites, with negligible fitness costs. The protective FREP1Q224 allele can be efficiently driven into FREP1L224 mosquito populations using a novel linked allelic-drive system that selectively targets the L224 codon replacing it with the parasite refractory Q224 allele. This anti-malarial drive system provides a novel genetic approach for confinable population modification to aid in eradicating malaria.

175 **GENIE grants new tools: GCaMP8 for calcium imaging and rubyACR to inhibit neuronal activity** Daniel Bushey[1], Hiroshi Shiozaki[1], Yichun Shuai[1], Jihong Zheng[1], Jeremy Hasseman[1], Ilya Kolb[2], Glenn Turner[1] [1]Janelia Research Campus, [2]Arcadia Science

The genetically-encoded indicator and effector (GENIE) project team at the HHMI Janelia Research Campus develops new optogenetic tools to use in Drosophila melanogaster. Two recent tools include jGCaMP8 and RubyACRs. jGCaMP8, the latest iteration in the GCaMP calcium sensor series, offers faster kinetics and an improved signal-to-noise ratio over its predecessor, jGCaMP7. However, the first jGCaMP8 lines produced exhibited lower expression than jGCaMP7. Here we show that adding back the RSET tag improves expression levels of jGCaMP8, while retaining its fast kinetics and high signal-to-noise.

GENIE also tested whether recently identified red-shifted anion channelrhodopsin (RubyACR) from Labyrinthulomycetes can hyperpolarize neurons in Drosophila melanogaster. Red illumination is advantageous because melanogaster vision is less sensitive to longer wavelengths and penetrates tissue deeper than shorter wavelengths. Electrophysiological recordings in motor neurons expressing two different RubyACRs, A1ACR1 and HfACR1 tagged with EYFP, detected strong hyperpolarization during illumination with blue, green, and red wavelengths. Compared to GtACR1, a green-activated ACR, RubyACR responses were faster at onset and offset, and larger in amplitude. Using red light to stimulate RubyACR while imaging activity with GCaMP revealed evidence for inhibition in Mi1 neurons of the visual system. Illuminating freely behaving animals expressing RubyACRs in glutamatergic neurons with either blue, green, or red wavelengths consistently inhibited spontaneous locomotion. To test whether RubyACR activation may actually drive release of synaptic vesicles, as observed in the mammalian brain, we examined whether light activation in the fly's dopaminergic neurons could provide effective reinforcement in an associative learning task. Although the cation-selective opsin CsChrimson effectively induced memory formation in this assay, RubyACR activation did not, suggesting it did not elicit dopamine release. Additionally, we show that RubyACRs can rapidly and reversibly inhibit ongoing courtship song, when expressed in the song descending neurons. Together, these findings demonstrate that RubyACRs are a reliable tool to inhibit neuronal activity in Drosophila.

176 ***In-silico* discovery of inter-organ communication proteins** Justin A Bosch, Carlie Widdison Human Genetics, University of Utah

Circulating hormones that bind receptors play key roles in inter-organ communication, but their discovery using traditional experimental approaches has been slow and incremental. Recent advances in AI-driven protein structure modeling now enable *in-silico* prediction of protein-protein interactions (PPIs), facilitating large-scale, high-throughput screens for novel hormone-receptor pairs. Despite this progress, *in-silico* screens are computationally prohibitive, requiring expensive processors (e.g. GPUs) and long timescales (e.g. months). Here, we describe an optimized *in-silico* PPI screening pipeline using AlphaFold at a high-performance computing (HPC) data center, which dramatically reduces computation time, expense, and manual intervention. A key component of these optimizations is tailoring computational resources based on protein size. We used this pipeline to screen more than 100,000 pairs of *Drosophila* circulating proteins versus cell surface proteins from 16 organ-organ axes (e.g. fat body to muscle), uncovering several hormone-receptor candidates. One top hit is an uncharacterized protein from larval fat body that we name "Adiposyn", which is secreted into hemolymph and is predicted to bind synaptic Neuroligin proteins. Overall, this *in-silico* screen provides the first step toward an unprecedented systems-level view of inter-organ communication. Our optimized pipeline can be easily adapted for other HPC data centers, PPIs, and species, enabling more scientists to discover *in-silico* protein-protein interactions at scale.

177 **Cellular programs enabling immune cell tissue invasion and beyond** Daria Siekhaus University of California Los Angeles

The immune system's capacity to destroy infectious agents or tumors depends on individual cells' ability to move through tissue barriers. On the protective side, macrophages enter tissues already during development to take up residence and regulate homeostasis, metabolism, and repair throughout life. We utilize the developmental migration of embryonic *Drosophila melanogaster* macrophages as they penetrate the extended germband to reveal what cellular programs are required in invading cells and invaded tissues to enable this infiltration. We have identified cell division as the main mechanism by which surrounding tissues controls macrophage entry, and have shown that this is conserved in vertebrates. We have also identified a specialized cell fate program for the leading macrophages which guides and enables tissue entry, and a requirement in these leaders for boosts in mitochondrial energy production. The programs we identify in invasion also can act in other tissues; the protein we identified as affecting mitochondria is linked in human GWAS studies to Alzheimer's, and affects neural cell death in *Drosophila* models of the disease. I will talk about our latest results on these topics.

178 **Interconversion of compound eyes and ocelli through the dialing of transcription factor levels** Justin Kumar Indiana University

The adult visual system of *Drosophila melanogaster* is composed of two compound eyes, a pair of extra retinal eyelets, and a trio of simple eyes called ocelli. These distinct eye types have evolved independently from each for over 400-500 million years and are anatomically distinct, connect to different regions of the brain, and control unique arrays of visual and circadian behaviors. The fate of compound eyes and ocelli is controlled by the activity of the Paired Box-6 (Pax6) transcription factors Eyeless (Ey) and Twin of Eyeless (Toy). Pax6 plays prominent roles in the developing eye of all seeing animals with strong loss-of-function mutants being associated with microphthalmia or anophthalmia-like phenotypes in humans, mice, zebrafish, and the fruit fly. Pax6 is also characterized by its ability to reprogram the fate of entire cell populations – the forced expression of Ey and Toy can induce the formation of ectopic eyes within non-ocular tissues. Ey and Toy differentially control the fate of the compound eyes and ocelli with the former specifying the identity of the compound eye and the latter directing the fate of the ocelli. These proteins appear to regulate a common set of downstream targets but activate them at vastly different levels within the two visual organs. This is due to Ey having a much stronger transcriptional activation domain than Toy. Quite surprisingly, simply modulating the levels or activity of Ey/Toy downstream targets induces a dramatic reprogramming of the ocelli into compound eyes. One target gene, *sine oculis* (*so*), encodes a DNA binding protein that can function as a transcriptional activator when bound to Eyes Absent (Eya) or a repressor when bound to Groucho (Gro). Our findings suggests that the stochiometric balance between So-Eya and So-Gro complexes is critical for selecting between the two different types of visual organ fates. Finally, ratcheting down levels of Ey and Toy (as well as their downstream targets) within the developing compound eye results in the reverse fate change and induces its conversion into ocelli, antennae, maxillary palps, and head epidermis. Our results suggest that adjusting the expression levels of field-level selector genes across a wide threshold range is a viable mechanism for specifying a diversity of cell and organ fates.

179 **Everything you ever wanted to know about sex (but were afraid to ask)** Mark Van Doren Johns Hopkins University

Mother nature is surprisingly sexually adventurous and the "switches" for deciding an embryo's sex vary widely in the animal kingdom. In flies, the key switch is the RNA binding protein Sex lethal (Sxl), which is expressed only in females and regulates both sexual identity and X chromosome dosage compensation. However, this mechanism is poorly conserved in insects. Similarly, the key switch in humans, the Y chromosome encoded gene SRY, is not conserved in all mammals. Yet this does not mean that nature uses completely different mechanisms for regulating sex-specific development; conserved members of the Doublesex, MAB-3 Related Transcription Factor (DMRT) family act downstream of different sex determination switches to control sexual dimorphism across the animal kingdom, from planaria to mammals, particularly in the developing gonads. Patients with mutations in human DMRT1 are unable to form testes, and exhibit gonad dysgenesis, infertility and sex reversal. Therefore, a main focus of our lab is to use Drosophila to understand how DMRTs regulate the formation of an ovary vs. a testis. The sex determination switches discussed above act in somatic cells, but sexual identity is also critical in the germline to enable formation of male and female germline stem cells, and production of sperm or eggs. Germline sex determination is controlled partly by signals that the germ cells receive from surrounding somatic cells. In addition, Sxl is also expressed only in XX germ cells, and is required for female identity in the germline. However, the way in which Sxl becomes activated, and the targets for regulation by Sxl in the germline, are different from in the soma. Therefore, another main focus of our work is to understand how signals from the soma combine with Sxl activity in the germ cells to control germline sexual identity and development. I will present an overview of how our thinking about sex determination in flies and other animals has changed in recent years along with describing some of the current work from our lab.

180 **Lipid droplets as regulators of oogenesis and embryogenesis** Michael Welte Biology, Univ Rochester

During animal development, the metabolism of individual stages and tissues has to be fine-tuned so that the appropriate molecules are available to build specific structures, to support growth or differentiation, or generate critical signals. Emerging evidence suggests that a critical player in these processes are lipid droplets, the cellular sites for fat storage. They exist in essentially all cell types and are essential regulators of lipid metabolism and energy homeostasis. It is also increasingly recognized that they play important roles in the life cycles of many proteins. We discovered that during mid-oogenesis lipid droplets mediate at least two distinct aspects of lipid metabolism needed for proper oocyte development. First, lipid droplets control fatty acid trafficking to mitochondria. If fatty acid release from lipid droplets is impaired, fatty acid oxidation in mitochondria is largely abolished, leading to a drop of mitochondrial membrane potential. If fatty acids cannot be sequestered by lipid droplets, they flood into mitochondria, causing excessive ROS and inducing apoptosis. Second, arachidonic acid stored as triglycerides in lipid droplets is critical for the production of prostaglandins, potent signaling lipids. These prostaglandins in turn mediate the remodeling of the actin the cytoskeleton which drives follicle morphogenesis. Lipid droplets also control development via their ability to regulate proteins. We discovered that during oogenesis lipid droplets sequester certain histones, via direct physical interactions with the anchoring protein Jabba. This sequestration prevents histone degradation and allows the mother to build up a large pool of excess histones to support embryogenesis. In pre-cellularization embryos, newly translated histone H2Av is transiently sequestered to lipid droplets, a process that regulates H2Av import into nuclei, H2Av incorporation into chromatin, and ultimately zygotic gene expression. These studies led to the discovery that levels of H2Av are a critical timer of a subset of events in the early embryo, including the degradation of a large fraction of maternal mRNAs. Our findings suggest that lipid droplets can control development via multiple, distinct mechanisms.

181 **Nutritional Inheritance in Early Development and Disease** Monica Dus[1], Manaswini Sarangi[2] [1]MCDB, University of Michigan, [2]University of Michigan

The dietary histories of grandparents and parents shape health and disease in subsequent generations. While some of this impact arises from molecular memories echoing across genomes, a significant part stems from a more direct and tangible inheritance: the nutrients provided by parents at the very start of existence. From the moment we become the first cell of ourselves, these inherited nutrients fuel, build, and guide the foundational processes of early life. This lecture will highlight our lab's efforts to characterize the nature of nutritional inheritance and examine its effects on development and lifelong health trajectories.

182T **A single-nucleus transcriptome atlas of the *Drosophila* embryogenesis to identify Jagunal as a regulator in early development** Stephanie Uzordinma Awuzie, Blake Riggs Cell and Molecular Biology, San Francisco State University

Asymmetric cell division is a highly conserved process across all species, essential for the generation of diverse cell types. Defects in this process during early development are linked to diseases stemming from imbalanced cell heterogeneity. In *Drosophila* embryogenesis, this form of cell differentiation occurs at the start of gastrulation, where proneural cell clusters are established and proliferate due to cell signaling. However, the mechanism by which proneural cell division initiates upstream signaling that influences asymmetric cell division remains unclear. Previous studies suggest that the Endoplasmic Reticulum (ER) may play a role in proneural cell fate, exhibiting unequal inheritance at the onset of gastrulation. Confocal imaging shows asymmetrical partitioning of the ER during prometaphase in an anterior region of the embryo, responsible for brain formation. This asymmetric inheritance of the ER depends on the highly conserved ER transmembrane protein, Jagunal (Jagn). Jagn has been previously characterized for its role in ER reorganization in *Drosophila* oocyte development, indicating its critical function in early development. However, Jagn's role in proneural division and the generation of cell diversity is poorly understood. Defects in Jagn expression are associated with defects in mitotic spindle rotation within the delaminating neuroblast, a process regulated by the proper localization of apical and basal cell fate determinants. This suggests that Jagn is involved in regulating the segregation and localization of cell fate determinants. By employing single-cell resolution transcriptomics, we can analyze Jagn's transcriptional activity throughout embryogenesis by using time-sensitive analysis. In proneural cells, Jagn is co-expressed with key developmental factors essential for brain formation in *Drosophila*. Furthermore, enrichment profiling of Jagn-expressing cells reveals down-regulation of genes associated with tissue polarity and cell differentiation, supporting Jagn's proposed function. Understanding this mechanism of cell differentiation provides insights into the role of JAGN1, the human homolog of Jagn, in diseases linked to disrupted cell differentiation, such as neutropenia, an immunocompromising blood condition.

183T **Apoptotic signaling pathways mediate the production of STING-dependent extracellular vesicles from malignant tumors in *Drosophila melanogaster*** Alexandra Fernandes[1], Jiae Lee[2] [1]California State University, Long Beach, [2]Biology, California state university long beach

Extracellular vesicles (EV), are emerging as an intracellular mediator that can also transport cellular material. Recently, malignant tumors have been shown to produce EVs, but the molecular mechanisms for their production and their function in tumor-host communication is not well known. We use a developing eye disc from the *Drosophila* larvae to induce Ras^{V12}, $scribble^{-}$ tumors and study their EVs in vivo. We have previously reported that Ras^{V12}, $scribble^{-}$ tumors produce EVs via cGAS-STING signaling, and they interact with the hemocytes, circulating blood cells, to then produce a systemic immune response in the fat body. In this study, we investigate the signaling pathways that mediate cGAS-STING signaling to EV production. We found that the markers for the apoptotic signaling pathway, phospho-JNK and cleaved-Caspase 3, in the Ras^{V12}, $scribble^{-}$ tumors are elevated from the contribution of undead tumor cells. The knock-down of STING by RNAi can revert the elevation of these apoptotic signaling. Moreover, the production of EV formation has decreased when apoptotic signaling becomes inhibited. These results suggest that STING-dependent EV formation in the Ras^{V12}, $scribble^{-}$ tumors is mediated by the apoptotic pathway.

184T **The role of asymmetric cell division in multilineage blood cell development during homeostasis in *Drosophila*** Gerson Ascencio[1], Lauren Goins[2] [1]Stanford University, [2]Developmental Biology, Stanford Univeristy

The Drosophila lymph gland is a great model for studying blood cell development and fate selection. The developing third-instar lymph gland is divided into three major zones: the medullary zone (MZ), composed of undifferentiated, progenitor cells, the cortical zone (CZ) populated by mature hemocytes comprising plasmatocytes, crystal cells, and lamellocytes, and the posterior signaling center (PSC). The PSC is a stem-cell niche which provides signals to the nearby MZ. However, the precise regulatory mechanisms governing the critical decision of precursor cells to halt proliferation and initiate terminal differentiation at the MZ-CZ border remain elusive. Preliminary data from our lab have revealed intriguing evidence suggesting that the Par protein complex, which typically orchestrates apical-basal polarity in epithelial cells and asymmetric distribution of determinants leading to asymmetric cell division (ACD) in neuronal stem cells in Drosophila, exhibits asymmetric localization in precursor cells. This asymmetry manifests specifically at the outer margin of the MZ preceding divisions that yield differentiating progeny in the Drosophila blood cell lineage. Here, we hypothesize that the Par polarity complex governs asymmetric cell division and the localization of cell fate determinants in hematopoietic progenitors. Our findings demonstrate that Par-6, a member of the Par complex, is asymmetrically distributed in some mitotic cells along the MZ-CZ boundary of developing third-instar larvae. Furthermore, loss of Par-6 disrupts cell fate determination in a subset of differentiating cells. These data suggest that the Par polarity complex plays a role in establishing proper polarity orientation in the developing lymph gland which is a critical step in stem cell fate determination and differentiation and plays a vital role in maintaining the stem cell microenvironment.

185T **Polarized trafficking of cell-cell adhesion proteins facilitates collective migration during embryonic wound healing** Sofia Mendez-Lopez[1], Kate MacQuarrie[1], Rodrigo Fernandez-Gonzalez[1,2], Katheryn Rothenberg[1,3] [1]University of Toronto, [2]Hospital for Sick Children, [3]University of Iowa

Collective cell movements drive the formation and repair of tissues during development and contribute to the spread of metastatic disease. To understand how cells coordinate their migration, we investigate wound healing in the *Drosophila* embryonic epidermis. Upon wounding, a supracellular cable composed of the cytoskeletal protein actin and the molecular motor non-muscle myosin II assembles around the wound, generating forces to coordinate cell movements and drive wound closure. The actomyosin cable forms through the polarization of actin and myosin in the cells adjacent to the wound. In parallel, adherens junction proteins, including E-cadherin, are depleted from former bicellular junctions at the wound edge and accumulate at former tricellular junctions around the wound (wTCJs). The reorganization of cell-cell adhesions is required for rapid wound healing. E-cadherin is removed from former bicellular junctions via endocytosis, but it is unclear how E-cadherin is delivered to wTCJs. Using photobleaching experiments, we found that E-cadherin diffusion along lateral junctions does not significantly contribute to wTCJ reinforcement. Instead, we found that a fluorescently tagged form of the small GTPase Rab11, implicated in vesicular trafficking, accumulated around the wound. Thus, we hypothesized that E-cadherin is delivered to wTCJs via protein transport pathways. To examine the potential role of vesicular trafficking in wound healing, we manipulated the activity of the small GTPases Rab11 and Rab4, which mark vesicles for slow and fast endocytic recycling, respectively. Reducing Rab11 activity by overexpressing a dominant-negative form slowed wound closure by 29% and reduced E-cadherin accumulation at wTCJs by 33%. Similarly, overexpressing a dominant-negative form of Rab4 slowed wound repair by 39% and reduced E-cadherin accumulation at wTCJs by 35%. Rab11 and Rab4 manipulations did not affect myosin polarization to the wound edge. Together, our results indicate that vesicular trafficking, potentially via endocytic recycling, contributes to the redistribution of E-cadherin during wound repair, and that cell-cell adhesion rearrangements control the rate of wound healing independent of cytoskeletal polarization.

186T **OA/Oamb through Gγ1, Cyst and RhoGEF2 in the follicular epithelium regulates Rho1-mediated contraction for follicle rupture during *Drosophila* ovulation** Stella E Cho, Jianjun Sun Physiology and Neurobiology, University of Connecticut

Drosophila ovulation releases a mature oocyte housed within a sac of epithelial follicle cells (known as a mature follicle) through a process called follicle rupture as in mammals. Our previous work demonstrated that octopamine (OA) and octopamine receptor in the mushroom body (Oamb) activate Rho1 GTPase-mediated actomyosin contraction in the follicle cell cortex to generate the mechanical force to facilitate the follicle rupture. However, the detailed signaling pathway downstream of Oamb, a G-protein-coupled receptor (GPCR), has not been explored. Here, we discovered that Gγ1, one component of the heterotrimeric G protein, is essential for the cortical enrichment of both Rho1 and phosphorylated non-muscle myosin II (NMM II; active NMMII) in response to OA signaling. In addition, Gγ1 knockdown caused defects in OA-induced follicle rupture *ex vivo* and ovulation *in vivo,* suggesting that non canonical $G_{\beta\gamma}$ may play a critical role downstream of Oamb to induce Rho1 activation and follicle rupture. Furthermore, we found that two guanine nucleotide exchange factors (GEFs), cyst and RhoGEF2, may play redundant roles in activating Rho1 in follicle cells after OA stimulation. Simultaneous depletion of both cyst and RhoGEF2 was necessary to disrupt the cortex enrichment of Rho1 and NMM II, while knockdown of either one alone showed minimal defect. Interestingly, double knockdown of both cyst and RhoGEF2 resulted defects only in OA-induced follicle rupture *ex vivo* but did not significantly impact egg-laying behavior *in vivo*. This implies that other compensatory mechanism may exist *in vivo* to ensure the robustness of egg laying. All these results led us to propose that OA/Oamb signaling activates $G_{\beta\gamma}$, which may further activate cyst and RhoGEF2 to induce Rho1 activation and actomyosin contraction. More work in on the way to thoroughly characterize this pathway in mature follicle cells. Given the conserved nature of GPCRs and Rho1, this work could illuminate the G protein signaling cascade involved in mechanical force generation for follicle rupture in other species or for other cellular processes.

187T **A new regulatory dimension of nuclear-cytoplasmic transport through the nuclear pore complex in early *Drosophila* embryos** Yuki Shindo, Soumya V Kumar The University of Texas at Dallas

Communication between the cytoplasm and the nucleus is governed by the transport of macromolecules across the nuclear envelope through the nuclear pore complex (NPC). While the NPC has been thought to be relatively invariant across cell types because of its essential housekeeping role, emerging evidence suggests that the NPC exhibits significant variation in number, post-translational modifications, and composition in different cellular and developmental contexts. In line with this, we have recently found that the amount of NPCs decreases during early embryogenesis in *Drosophila*, Here, we leverage the early embryo system to interrogate the mechanistic basis for how differences in NPC state feed into cellular and developmental processes. Using genetics, genome editing, live imaging, and synthetic reporter cargoes, we dissect the effects of changing NPC numbers on nuclear-cytoplasmic transport and early embryonic development. We propose a model in which the NPC is not a mere housekeeping apparatus but an active regulator of cell and developmental biology. In addition, we extend this concept to the regulation of transport receptors, other key players in nuclear-cytoplasmic transport, and discuss a non-canonical role for the nuclear export signal (NES) beyond nuclear export control.

188T **Mechanisms of TNF-α Pathway Activation in Response to Gliotactin Overexpression in Wing Disc Epithelia** Zazil Adriana Solis Saldivar, Vanessa Auld University of British Columbia

Epithelial cells establish and maintain permeability barriers. These barriers form between adjacent cells, called septate junctions (SJs), and at sites where three cells meet, called tricellular junctions (TCJs). Both SJs and TCJs are comprised of specialized protein complexes that are tightly regulated in mammals and *Drosophila melanogaster*. However, the mechanisms that regulate them remain poorly understood. Misregulation of these junctional proteins can disrupt epithelial tissue homeostasis, potentially contributing to cancer development. When Gliotactin (Gli), a *Drosophila* TCJ protein, is overexpressed in wing imaginal disc epithelia, it spreads away from the TCJs, resulting in deleterious phenotypes such as apoptosis, over-proliferation, delamination and resulting cell migration. Our data shows that the TNF-α signaling pathway mediates these phenotypes through the JNK pathway. The TNF receptor, Grindelwald (Grnd), mediates the pro-apoptotic functions of Eiger (Egr), the unique *Drosophila* TNF ligand. Downregulation of Grnd and downstream signaling components suppresses the overexpression phenotypes of Gli, and Grnd becomes internalized in Gli positive endocytic vesicles. Binding of Eiger and endocytosis of Grnd is necessary for receptor activation. While RNAi-knockdown of Eiger in the wing disc did not suppress the Gli phenotypes, complete and systemic loss of Egr did, supporting that Grnd mediates Gli overexpression phenotypes in a ligand-dependent manner. In normal epithelia, Grnd and Egr are physically separated by the permeability barrier created by the SJs and TCJs. Our data shows that the barrier is intact when Gli is overexpressed while apoptosis is blocked using a dominant negative JNK or a Grnd mutant. This means that excess Gli does not disrupt the permeability barrier, raising the question: how are Grnd and Egr seeing each other when Gli is overexpressed? Grnd is localized in the apical region of the cell while Egr is secreted and present basally, leading to the hypothesis that the spread of Gliotactin causes the mislocalization of Grnd basal to the SJs leading to ectopic interactions with Egr. I will test this hypothesis by assessing the distribution of Grnd, Gli, and Egr in the epithelia to determine if the spread of Gliotactin leads to the presence of Grindelwald basal to the permeability barrier. This research could establish a link between the TNF-α pathway, apoptosis, and TCJ protein misregulation in *Drosophila*.

189T **Mushroom bodies tiny regulates Sidekick localization to tricellular adherens junctions** Jessica E Treisman[1], Dhaval Gandhi[1], Brian Griffin[1], Maria Bustillo[1], Genie Jang[1], Ariel Hairston[2] [1]Cell Biology, New York Univ Med Ctr, [2]Program in Neuroscience, Harvard University

The homophilic cell adhesion molecule Sidekick (Sdk) acts at tricellular adherens junctions (tAJs) to control cell bond tension and facilitate cell rearrangements. Sdks are also localized to specific synaptic layers in the *Drosophila* optic lobes and vertebrate retina. It is not known how these distinctive patterns of protein localization are generated. We have used CRISPR to do a structure-function analysis of endogenous Sdk. Sdk can only localize to tAJs if it is present in at least two of the three cells, suggesting a role for homophilic adhesion, which is mediated by the first four immunoglobulin (Ig) domains of mammalian Sdks. Surprisingly, deleting these domains does not alter the localization of *Drosophila* Sdk to tAJs, although deleting the fifth and sixth or all six Ig domains, or the thirteen fibronectin type III domains, abolishes this localization. Deleting the C-terminal PDZ-binding motif of Sdk, which mediates its interactions with the PDZ proteins Canoe (Cno), Polychaetoid and dGIPC, slightly reduces the tAJ enrichment of both Sdk and Cno. A yeast two-hybrid screen revealed that the intracellular domain of Sdk also directly binds to the protein kinase Mushroom bodies tiny (Mbt). Sdk enrichment at tAJs is strongly reduced in *mbt* mutant clones, and its localization is rescued by a wild-type *UAS-mbt* transgene but not by one lacking Mbt kinase activity. In addition, a constitutively active form of Mbt can mislocalize Sdk along bicellular junctions. Although the intracellular domain of Sdk contains predicted Mbt phosphorylation sites, mutating two of these does not alter its localization or prevent it from being mislocalized by activated Mbt. Mbt activity may regulate Sdk localization indirectly, perhaps by changing cell bond tension. Finally, none of the manipulations we have tested alter Sdk localization to specific synaptic layers in the optic lobes; it is possible that this pattern is achieved by transport and local translation of *sdk* mRNA. These findings reveal an important role of Mbt, a homologue of human Pak4, in establishing specialized structures at tAJs.

190T **The Regulation of Cell Fate Determinants by the microRNA, miR-190, During Asymmetric Cell Division in Drosophila Neuroblasts** Laura Galvan[1], Gerson Ascencio[2], Blake Riggs[1] [1]Biology Department, San Francisco State University, [2]Institute for Stem Cell Biology and Regenerative Medicine, Stanford University

Asymmetric cell division (ACD) is fundamental to neurogenesis in *Drosophila melanogaster*, where neural stem cells, or neuroblasts, divide to produce one self-renewing stem cell and one differentiating daughter cell, ensuring a balance between stem cell maintenance and neuronal differentiation. This process heavily depends on the establishment of apical-basal polarity, orchestrated by a set of conserved polarity proteins, including atypical protein kinase C (aPKC), Partitioning defective 6 (Par6), Scribble (Scrib), Lethal giant larvae (Lgl), and Discs large (Dlg). Within neuroblasts, the PAR complex (Par3, Par6, and aPKC) occupies the apical cortex to establish polarity, while Scrib, Lgl, and Dlg localize to the cortex to confine apical components. Proper localization and function of these polarity proteins are critical for ACD, maintaining cell polarity, and preventing tumor-like overproliferation, however, it is unclear how polarity is established and regulated. Emerging research suggests that microRNAs (miRNAs) play significant roles in post-transcriptional gene regulation, influencing ACD and cell fate determination in neuroblasts. These small non-coding RNAs bind to the 3’ untranslated region (3’-UTR) of target mRNAs, resulting in translational repression or mRNA degradation. Through our research, we identified miR-190 as a potential regulator of neuroblast cell polarity. Using the computational tool TargetScanFly, we discovered highly conserved binding sites for miR-190 in the 3’-UTRs of several polarity and cell fate determinants, including Scribble and Prospero, suggesting that miR-190 modulates their expression. Preliminary data show that miR-190-deficient embryos exhibit altered mRNA levels for key cell fate determinants, with Prospero mRNA upregulated nearly two-fold. Immunofluorescence and high-resolution confocal microscopy reveal mislocalization of Prospero, Scribble, Miranda, Bazooka, and aPKC, indicating disruptions in cell polarity and ACD. We hypothesize that miR-190 regulates the localization and function of cell polarity proteins by targeting their mRNAs, thus ensuring proper asymmetric division and cell fate selection. This research aims to delineate the role of miR-190 in regulating polarity proteins and maintaining the neuroblast’s intrinsic ability to divide asymmetrically. Our findings will enhance the understanding of the molecular mechanisms underlying cell polarity and fate determination, underscoring the significant role of miRNAs in neurogenesis and cellular organization during neuroblast division.

191T **The role of the repetitive region of *Hsr-omega* long non-coding RNA in the formation of B-bodies.** Sharar Haque, Anton Bryantsev Kennesaw State University

Nuclear domains are distinct, well-defined compartments that selectively accumulate nuclear proteins, though their regulatory mechanisms remain largely unclear. The B-body is a specialized nuclear domain composed of RNA-binding proteins, including splicing regulators such as Bruno and Hrb87F. Previous work in our lab identified the long non-coding RNA *Hsr-omega* as a scaffolding molecule of B-bodies, and here we investigate which *Hsr-omega* regions are essential for B-body assembly. The *Hsr-omega* genetic locus produces various transcripts, ranging in size and composition. The long *Hsr-omega* isoforms detected in B-bodies include a 10-kb repetitive region (RR) with multiple imperfect direct repeats. Using bioinformatics tools, we found that the RR is enriched in binding sites recognized by the RNA-binding domains of Bru and Hrb87F. To probe the role of this region in B-body organization, we overexpressed the RR-derived 277-bp core sequence (RR-core) and tracked its localization via *in situ* hybridization (FISH). Expression of RR-core was evident through prominent transcription sites, but RR-core transcripts did not accumulate at B-bodies, as they were effectively exported to the cytoplasm. Immuno-FISH observations showed that neither Bru nor Hrb87F accumulated at RR-core transcription sites. These results suggest that immobilization of nuclear RNA and/or the presence of multiple binding sites may be required to recruit protein components to nuclear domains. *Hsr-omega* and its interacting proteins provide a valuable model for studying nuclear domain formation.

192T **Actin modification by Mical/SelR is necessary for actomyosin ring dynamics and efficient cell wound repair** Mitsutoshi Nakamura, Cassandra Aarrestad, Susan M Parkhurst Basic Sciences Division, Fred Hutchinson Cancer Center

Individual cells within tissues and organs are subjected to damage caused by daily wear-and-tear and environmental/physiological stresses. To survive this damage and remain functional, cells have a robust repair mechanism comprised of rapid membrane resealing/remodeling and dynamic cytoskeletal repair at the cell cortex that are initiated by calcium influx. Actomyosin ring contraction at the wound edge is one major mechanism that generates the physical force for wound closure. We recently found that Rab35 is recruited to wounds and its RNAi knockdown disrupts actomyosin ring assembly and disassembly in the *Drosophila* cell wound repair model. Rab35, a member of Rab family GTPases, regulates membrane trafficking and cytoskeleton dynamics in many cellular processes. In particular, Rab35 regulates Mical, a Redox enzyme that binds to F-actin and promotes F-actin clearance during cytokinetic abscission. SelR is a methionine sulfoxide reduction enzyme that works as a counterpart to Mical. We find that both Mical and SelR are recruited to cell wounds. Unexpectedly, both Mical and SelR RNAi knockdowns exhibit a disrupted actomyosin ring at the wound edge. To further examine the redox reaction of actin during cell wound repair, we generated a transgenic line having a point mutation on Methionine 44 that is known to be oxidized by Mical. Consistent with Mical knockdown, the point M44 mutation impairs actomyosin ring formation and efficient cell wound repair. Our results suggest that actin modifications play a mechanistic role in the formation of the actomyosin ring and efficient cell wound repair.

193T **Exploring the functional interaction of Kibra and aPKC in the Follicular Epithelium** KathyAnn Lee[1], Richard Fehon[2] [1]Committee on Development Regeneration and Stem Cell Biology, University of Chicago, [2]Molecular Genetics and Cell Biology, University of Chicago

The highly conserved Hippo signaling pathway coordinates tissue growth with morphogenesis in animal development. Hippo signaling also has a complex relationship with apical-basal polarity in epithelial cells - disruptions in Hippo signaling components result in a larger apical domain and disruptions of polarity components affect Hippo signaling. Interestingly, Kibra, an upstream regulator of Hippo signaling, is known to interact with aPKC, a key apical polarity protein, via a substrate motif, suggesting that Kibra could be a focal point for Hippo-apical polarity interaction. Previous work in mammalian cells suggests that Kibra inhibits aPKC's kinase activity, though this has not yet been demonstrated in vivo. Conversely, recent work in our lab indicates that aPKC represses Kibra's ability to promote Hippo signaling. We are studying the relationship between Kibra and aPKC, and how this relationship intersects with cell polarity, the cytoskeleton, and Hippo signaling. Using the follicular epithelium, which has been used extensively to study epithelial polarity and Hippo pathway function, we are examining the effects of gain and loss of Kibra function on follicle cell polarity and migration. Notably, ectopic Kibra expression leads to abnormal cell shape and loss of epithelial integrity. This effect is enhanced by decreased aPKC dosage, suggesting that in Drosophila Kibra can antagonize aPKC function. Further studies will seek to better understand how Kibra controls aPKC function. Additionally, the follicular epithelium provides a powerful system in which to continue our functional studies of how aPKC sequesters Kibra to the junctional cortex and represses Kibra function.

194T **Vitellogenic oocytes is an excellent in vivo moderl to study endolysosomal trafficking in *Drosophila*** Stephen M. Farmer[1,2,3], Yue Yu[1,3], Dongsheng Chen[3], Shiyu Xu[3], Beatriz Rios[4], amanda solbach[1,3], Xin Ye[3], Lili Ye[3], Sheng Zhang[3,5,6] [1]Program in Neuroscience, The University of Texas MD Anderson Cancer Center UTHealth Graduate School of Biomedical Sciences (MD Anderson UTHealth GSBS), [2]Program in Biochemistry and Cell Biology, The University of Texas MD Anderson Cancer Center UTHealth Graduate School of Biomedical Sciences (MD Anderson UTHealth GSBS), [3]Institute of Molecular Medicine, The University of Texas Health Science Center at Houston (UTHealth), [4]Program in Neuroscience, The University of Texas Health Science Center at Houston (UTHealth), [5]Department of Neurobiology and Anatomy, The University of Texas Health Science Center at Houston (UTHealth), [6]Programs in Genetics & Epigenetics and Neuroscience, The University of Texas MD Anderson Cancer Center UTHealth Graduate School of Biomedical Sciences (MD Anderson UTHealth GSBS)

Endocytosis and endolysosomal trafficking are essential for almost all aspects of physiological functions of eukaryotic cells. As our understanding on these membrane trafficking events are mostly from studies in yeast and cultured mammalian cells, one challenge is to systematically evaluate the findings from these cell-based studies in multicellular organisms under physiological settings. One potentially valuable *in vivo* system to address this challenge is the vitellogenic oocyte in *Drosophila,* which undergoes extensive endocytosis by Yolkless (Yl), a low-density lipoprotein receptor (LDLR), to uptake extracellular lipoproteins into oocytes and package them into a specialized lysosome, the yolk granule, for storage and usage during later development. However, by now there is still a lack of sufficient understanding on the molecular and cellular processes that control yolk granule biogenesis. Here, by creating genome-tagging lines for Yl receptor and analyzing its distribution in vitellogenic oocytes, we observed a close association of different endosomal structures with distinct phosphoinositides and actin cytoskeleton dynamics. We further showed that Rab5 and Rab11, but surprisingly not Rab4 and Rab7, are essential for yolk granules biogenesis. Instead, we uncovered evidence for a potential role of Rab7 in actin regulation and observed a notable overlap of Rab4 and Rab7, two Rab GTPases that have long been proposed to have distinct spatial distribution and functional roles during endolysosomal trafficking. Through a small-scale RNA interference (RNAi) screen on a set of reported Rab5 effectors, we showed that yolk granule biogenesis largely follows the canonical endolysosomal trafficking and maturation processes. Further, the data suggest that the RAVE/V-ATPase complexes function upstream of or in parallel with Rab7, and are involved in earlier stages of endosomal trafficking events. Together, our study provides s novel insights into endolysosomal pathways and establishes vitellogenic oocyte in *Drosophila* as an excellent *in vivo* model for dissecting the highly complex membrane trafficking events in metazoan.

195T **Characterizing actin structures during tube formation in *D. melanogaster* egg chambers** Luana Paleologu[1], Celeste Berg[2] [1]Genome Sciences, University of Washington, [2]University of Washington

The folding of epithelial sheets, processes known as wrapping and budding, are the foundation for creating many tubular organs in nearly all multicellular organisms. Nevertheless, there is much to understand about the molecular mechanisms that drive cell shape changes and tissue folding. The Berg lab uses genetic and imaging techniques to understand tube formation in *D. melanogaster* egg chambers. The egg chambers form tubes that fill with chorion protein, creating eggshell structures called dorsal appendages that help adequately oxygenate embryos. These dorsal appendage-forming structures serve as a good model system to study tubulogenesis since the tissue folding and cellular shape changes during tube formation are well understood. Two fundamental processes create and shape the dorsal appendage tubes: wrapping and tube elongation. While we have a strong understanding of how cells are moving during these processes, we do not know how the cytoskeleton is coordinating shape changes, and by what signaling mechanisms these cytoskeletal dynamics are regulated. To assess these processes, we are using phalloidin-stained fixed tissue to characterize actin structures using confocal microscopy, as well as live imaging of actin to characterize dynamics. These approaches revealed dynamic basal actin throughout tube formation in addition to expected apical structures associated with apical constriction, biased apical expansion, and cell intercalation. In particular, supracellular stress fibers undergo disassembly during tube wrapping and reform as tube elongation progresses. Here we present a study of the changes in the actin cytoskeleton during dorsal appendage formation with a focus on how this remodeling drives cell shape change and movement. We hypothesize that the disassembly of stress fibers in the tube-forming cells is necessary for proper cell shape changes during wrapping, and reestablishment of stress fibers are necessary for tube elongation.

196T **Waldorf binds to apical β_H-spectrin to regulate apical domain stability and cell cycle-independent Crumbs/ Expanded/Hippo-Warts pathway-mediated growth** Kristen Browder[1,2], Seung-Kyu Lee[2,3], Elizabeth Klipfell[4], Bryce MacIver[2,5], Claire Thomas[2] [1]Genentech, Inc., [2]Biology, Penn State University, [3]Natonal Institute of Aging (NIH/NIA/ IRP), [4]Midview High School, [5]Beth Israel Deaconess Medical Center

Spectrin is a large F-actin crosslinking protein that famously forms 2D networks in association with the plasma membrane to determine the cell shape of red blood cells. In NON-erythroid tissues, spectrin has additional roles in the endomembrane system. We have previously shown that the apically polarized $\beta_H$spectrin, encoded by the *karst* locus in *Drosophila*, is required for the stability of several apical proteins by promoting recycling to the plasma membrane. 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. Reduction in the level of β_H results in oversized tissues attributed to HWP modulation via multiple pathways. The PP2A substrate-specificity subunit Waldorf (Wdf; a PP2A-PR72/B'' isoform) is a binding partner of $\beta_H$spectrin that binds to β_H *via* a conserved sequence in segment 33. Genetic interaction and molecular epistasis experiments indicate that $PP2A^{Wdf}$ 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 Wdf levels. Wdf knockdown reduces wing size consistent with a model in which $PP2A^{Wdf}$ normally acts in a homeostatic fashion to limit Crumbs activation of the HWP by displacing aPKC complex after endocytosis. Like β_H and its previously reported partner Annexin B9, Wdf is tied to protein trafficking and localizes to a late-endosomal compartment where its knockdown leads to an increase in Rab7-positive compartments. This suggests that $PP2A^{Wdf}$ also normally acts by suppressing lysosomal trafficking, most likely in favor of recycling pathways. Wdf knockdown has the opposite effects on wing size to β_H knockdown, suggesting that β_H is not simply scaffolding Wdf in the apical Crumbs complex. β_H knockdown increases cells size, at least partially accounting for its tissue overgrowth phenotype. Dominant negative expression of βH33 in the eye results in apoptosis and a rough eye phenotype. A suppression screen based on co-overexpression of loci using EP-lines identified the cell cycle regulator String/Cdc25. Interestingly, our initial results indicate that String can also act to suppress Crumbs overexpression, and that this is not dependent on the canonical G2/M function of String.

197T **Identification of the Wnt Maturation Complex** Kate M Henesey[1,2], Senel Tektas[2], Ah-Ram Kim[3], David L. Raden[2], Norbert Perrimon[4,5], Erica M Selva[2] [1]Molecular and Cellular Biology, Harvard University, [2]Biological Sciences, University of Delaware, [3]Genetics, Harvard Medical School, [4]Department of Genetics, Blavatnik Institute, Harvard Medical School, [5]Howard Hughes Medical Institute, Harvard Medical School

Wnt signal transduction is fundamental to human development and is responsible for maintaining cellular homeostasis later in life. During development, reduced or absent Wnt signaling results in a wide range of developmental defects and aberrant signaling is recognized as a primary driver of cancer. While the numerous Wnt ligands and target receptors in receiving cells have been extensively studied, the maturation of virtually all Wnt ligands within sending cells is relegated to highly conserved yet understudied components, Porcupine (Por) and Wntless (Wls). Initial characterization of Por and Wls function within *Drosophila* to produce functional Wnt has informed much of our understanding of Wnt ligand maturation. *Further characterization of the mechanisms by which these players interact to produce active Wnt ligand is essential to better delineate how Wnt signaling is regulated.*

Herein we provide identification and initial characterization of the Wnt Maturation Complex. We discovered that Wls forms homo-dimers dependent upon intermolecular disulfide bridge formation, and these Wls dimers interact with Wnt and Por (2:1, Wls-Wnt, Wls-Por). Remarkably, this provides a universal mechanism for Wls dimerization and a possible explanation for the uncharacterized Wnt hand-off between Por and Wls in early ER Wnt processing. The overarching goals of this work are two-fold: to characterize disulfide bonded Wls dimer interactions in forming Wnt maturation complexes and to characterize disulfide bonded Wls dimer importance in Wnt post-translational modification, secretion, and signaling. We have generated various tagged forms of *Drosophila* Por and Wls wild-type and conserved cysteine mutants that are used in co-immunoprecipitation experiments to examine the importance of Wls disulfide bonds in forming early Wnt maturation complexes. To determine if Wls cysteine mutants affect posttranslational modification, secretion, and signaling, we used readouts of Wnt N-glycosylation, secretion, and canonical Wnt signaling. We are currently performing IP-MS to validate the complex and identify additional players involved in Wnt Maturation Complex dynamics. *This work will lead to an understanding of how Wls dimers function within a cell to produce functional Wnt ligand, impacting our comprehension of Wnt cellular dynamics, processing, and secretion mechanisms.* Results from this work have the potential to identify targeted therapeutics for diseases fueled by aberrant Wnt signaling.

198F **CG14767: a novel regulator of the Hippo/Yki tumor suppressor pathway** Swastik Mukherjee, Emma Spies, Alexey Veraksa Biology, University of Massachusetts at Boston

The Hippo/Yorkie (Yki) tumor suppressor pathway is an evolutionarily conserved system in metazoans that controls cell proliferation, differentiation, and cell death. The Hippo pathway is regulated by endosomal trafficking; however, the underlying mechanism is poorly understood. In a screen for *Drosophila* Yki-interacting proteins, we identified a novel interactor, CG14767, that may be involved in endocytic regulation of Yki function. The mammalian orthologs of CG14767 belong to the lysosomal-associated protein transmembrane (LAPTM) family. They are localized in late endosomes and play multiple roles in mammalian cells like conferring multidrug resistance and promoting autophagy, thereby enhancing tumor cell growth. However, the function of *Drosophila* CG14767 is unknown. The goal of this work is to uncover the molecular mechanisms by which CG14767 controls Yki activity at the level of endosomal trafficking. Using protein-protein binding assays, we have shown that the binding between CG14767 and Yki is mediated via the L/PPxY motif/WW domain interaction. Genetic tests have revealed that unlike mouse LAPTMs, CG14767 prevents abnormal growth in *Drosophila*. Thus, CG14767 is both required and sufficient for limiting Yki-induced overgrowth of *Drosophila* wings and eyes. We have shown that CG14767 is involved in the endolysosomal trafficking, where it colocalizes with Yki in cultured *Drosophila* S2 cells. Further colocalization and protein-protein binding assays have revealed that CG14767 interacts with previously characterized endosomal proteins in the Yki network, Leash and Myopic (Mop). We hypothesize that CG14767 inhibits Yki activity either by sending it for degradation or by cytoplasmic retention. We are addressing this hypothesis by investigating the cellular and molecular mechanisms of CG14767 function using a combination of genetics and cell biological approaches. This research will expand our knowledge of the mechanisms of Yki regulation and will provide further insights into the regulation of Hippo signaling by the endolysosomal system.

199F **Organization of the apical extracellular matrix during tubular organ formation** SeYeon Chung[1], J. Luke Woodward[2], Jeffrey Matthew[2], Vishakha Vishwakarma[2], Ying Xiao[2] [1]Biological Sciences, Louisiana State University, [2]Louisiana State University

The apical extracellular matrix (aECM) plays a critical role in epithelial tube morphogenesis during organ formation, but its composition and organization remain poorly understood. Using the *Drosophila* embryonic salivary gland (SG) as a model, we identify Papss, an enzyme that synthesizes the universal sulfate donor PAPS, as a critical regulator of tube lumen expansion. *Papss* mutants show a disorganized apical membrane, condensed aECM, and disruptions in Golgi structures and intracellular trafficking. Additionally, we identify two zona pellucida (ZP) domain proteins, Piopio (Pio) and Dumpy (Dpy), as key components of the SG aECM. In the absence of *Papss*, Pio is gradually lost in the aECM, while the Dpy-positive aECM structure is condensed and dissociates from the apical membrane, leading to a thin lumen. Mutations in *dpy* or *pio*, or in *Notopleural*, which encodes a matripase that cleaves Pio to form the luminal Pio pool, result in a SG lumen with alternating bulges and constrictions, with the loss of *pio* leading to the loss of Dpy in the lumen. Our findings underscore the essential role of sulfation in organizing the aECM during tubular organ formation and highlight the mechanical support provided by ZP domain proteins in maintaining luminal diameter.

200F **Role of MICOS Complex in Mitochondrial Dysfunction During Aging** Blake Riggs, Victor D. Knowles, Stephanie Uzordinma Awuzie, Liam Kennedy Moore, Mikesha Carter, Laura Galvan Biology, San Francisco State University

Mitochondrial cristae support oxidative phosphorylation in the electron transport chain, generating ATP. The mitochondrial contact site and cristae organizing system (MICOS) are crucial in preserving cristae structure. However, how it influences mitochondrial stability across a cell's lifespan and its role in cellular aging remains poorly understood. We propose that loss of MICOS function leads to mitochondrial defects that drive age-related dysfunction. To investigate this, we utilized the model organism Drosophila melanogaster with a CRISPR/Cas9 system under the muscle-specific Mef2 promoter, creating a muscle-targeted knockout of the MICOS components mitofilin and the mitochondrial fusion gene Opa1. Mitofilin is crucial in maintaining cristae structure, while Opa1 regulates mitochondrial fusion. We assess functional decline through a negative-geotaxis assay, using climbing ability to indicate neuromuscular integrity with age. Muscle tissue provides insight into neuromuscular integrity and motor decline with age; however, examining the brain is essential due to high susceptibility to oxidative stress and reactive oxygen species (ROS) accumulation, critical indicators of aging and neurodegeneration. Immunohistochemistry and confocal microscopy will detect oxidative stress levels in MICOS-deficient brain tissue, marked by the intensity of the 8-OHdG DNA damage marker. Preliminary observations suggest a reduction in muscular and motor function in MICOS-deficient flies, highlighting the crucial role of mitofilin and Opa1 in maintaining cristae structure and ATP production. Increased oxidative stress in brain tissue suggests elevated ROS levels due to MICOS loss, potentially accelerating aging. Further testing through a lifespan assay will clarify the MICOS complex's impact on aging progression, which can develop strategies against age-related diseases.

201F **Elucidating the structural and functional roles of Sarcalumenin, a key component of the muscle fiber sarcoplasmic reticulum** Nechama Sasson[1], Tom Biton[1], Sergey Mursalimov[1], Ilan Zemski[1], Yael Alon[1], Dana Lorber[2], Talila Volk[2], Eyal Schejter[2], Ori Avinoam[1] [1]Biomolecular Sciences, Weizmann Inst Sci, [2]Molecular Genetics, Weizmann Inst Sci

The structural organization of a muscle fiber is critical to its function. This is particularly evident at the sarcomere level, where alternating bands of thin (actin) and thick (myosin) filaments mediate muscle contraction. A second example is the sarcoplasmic reticulum (SR), a muscle-specific variant of the endoplasmic reticulum (ER) and critical mediator of the process by which neuronal excitation is translated into muscle fiber contractility. The SR, which serves as a major store of Ca2+ ions, key mediators of excitation-contraction coupling, aligns with the sarcomeres along its longitudinal domain, while its junctional domain interfaces with the t-tubule indentations of the muscle fiber membrane.

Sarcalumenin (SRL), a relatively uncharacterized SR and muscle-specific protein, is recognized as an abundantly-expressed luminal Ca2+-buffering protein of the mammalian longitudinal SR. Intriguingly, SRL possesses a dynamin-like domain in addition to its Ca2+-binding domain, suggesting a parallel functional role in SR membrane remodeling. We have begun to characterize the single *Drosophila* homolog of SRL (CG9297), in order to complement and extend our studies of mouse SRL. Similar to its mammalian counterpart, *Drosophila srl* is expressed specifically in muscle fibers. Both homozygous disruption of the *srl* locus and muscle-specific knockdown result in larval lethality. Initial characterization of the *srl* mutant phenotype reveals defects in SR organization and Ca2+ homeostasis in larval body-wall muscles, while overall sarcomeric morphology appears intact. We plan to present the results of our ongoing efforts to elucidate the involvement of SRL in mediating larval muscle SR function and its contribution to maintenance of the structure of the SR reticular network. Key aspects of our findings on mouse SRL will be presented as well.

202F **Comprehensive Analysis of the Exocyst Complex in Regulated Exocytosis: Novel Roles and Pathway Requirements** Sofia Suarez[1], Sabrina M Fresco[1], Julián M Valinoti[1], Eleonora M Sorianello[2], Pablo M Wappner[1], Mariana Melani[1] [1]Fundación Instituto Leloir, [2]Instituto de Biología y Medicina Experimental

Using the larval salivary gland, we have characterized previously undescribed functions of the exocyst complex in regulated exocytosis. The salivary gland synthesizes mucins (Sgs proteins) that are packed in secretory granules (SGs) that sprout from the *trans*-Golgi network as immature SGs. These immature granules undergo a maturation process that involves homotypic fusion, acidification and acquisition of membrane proteins. After maturation, SGs fuse with the apical domain of the plasma membrane in response to a rise in ecdysone levels, releasing their content into the salivary gland lumen. The exocyst is an evolutionary-conserved hetero-octameric complex that participates in tethering of vesicles to the plasma membrane during constitutive exocytosis. By precise temperature-dependent gradual activation of the Gal4-UAS expression system, we have induced different levels of silencing of each of the eight exocyst complex subunits, and identified three temporarily distinctive steps along the exocytic pathway where the exocyst is critically required. We found that severe silencing conditions of any of the eight exocyst complex subunits results in Sgs retention in the endoplasmic reticulum and Golgi complex disorganization, indicating an early role of the complex in SG biogenesis. Moderate silencing of the complex allows SG biogenesis but blocks the pathway at maturation: we observed an accumulation of immature SG as indicated by its size and the failure to recruit maturation markers like Synaptotagmin-1 and CD63. Finally, very mild silencing of exocyst complex subunits results in formation of mature SGs that fail to fuse the plasma membrane since fusion markers such as $PI(4,5)P_2$ are not incorporated into SG membrane and SG content is not released into the gland lumen. Moreover, live imaging visualization of exocyst complex revealed that its localization is dynamic during salivary gland development and accompanies each of the proposed functions i.e.: the exocyst localizes at the trans-Golgi network during SG biogenesis, at immature SGs during maturation and at the contact sites between mature SG and the plasma membrane during SG-plasma membrane fusion. Our results shed light on previously unidentified functions of the exocyst along the exocytic pathway. We propose that the exocyst acts as a general tethering factor in various steps of this cellular process and that each of these functions relay on different levels of exocyst complex activity.

203F **Regulation of membrane-associated dense-core granule biogenesis in secretory compartments is controlled by Amyloid Precursor Protein-Like and disrupted by Aβ and Tau expression** Preman J Singh[1,2], Bhavna Verma[1], Adam Wells[1], Claudia Mendes[1], Dali Dunn[1], Ying-Ni Chen[3], Jade Oh[1], Lewis Blincowe[1], Mark Wainwright[1], Roman Fischer[1], Shih-Jung Fan[1,3], Adrian L Harris[1], Deborah CI Goberdhan[1], Clive Wilson[4] [1]University of Oxford, [2]US Dept of Veteran Affairs, Veterans Affairs Medical Center, [3]National Central University, [4]Univ Oxford

During maturation of regulated secretory compartments, signalling proteins aggregate into dense-core granules (DCGs) that dissipate upon secretion. The dynamic intra-compartmental events regulating this event, which frequently involves amyloid formation, have remained unclear, because most DCG compartments are barely resolvable by conventional light microscopy. By studying the maturation of the 5µm-diameter DCG compartments in prostate-like secondary cells (SCs) of the *Drosophila* male accessory gland, we have found that these compartments transition to Rab11-positive, recycling endosomal identity before forming both DCGs and intraluminal vesicles (ILVs) that are secreted as so-called Rab11-exosomes. Human cells have recently been shown to use the same mechanisms, contrary to long-established models for regulated secretion. Here we follow DCG assembly in real-time using a gene trap expressing GFP-tagged MFAS, the fly orthologue of human amyloidogenic protein TGFBI. We show MFAS is a major component of SC DCGs required for their assembly. Several genetic manipulations that affect ILV formation and/or the organisation of the DCG compartment's limiting membrane disrupt DCG biogenesis, suggesting that membranes are required for normal initiation of DCG protein aggregation. We find that Amyloid Precursor Protein-Like, APPL, the fly orthologue of human APP, is involved in this membrane priming event and its extracellular domain must subsequently be released by secretase cleavage at sites implicated in Alzheimer's Disease (AD) pathology for normal DCG maturation. These functions of APPL can be replaced by human APP, while expression of non-cleavable forms of APPL or secretase knockdown disrupts this process. Furthermore, expression of mutant Aβ-peptides associated with familial AD blocks release of DCG aggregates from ILVs and the limiting membrane, leading to lysosomal targeting of DCG compartments. Aβ-peptide expression also induces abnormal endocytosis of secreted membrane-associated aggregates by other cells, transferring endolysosomal defects to non-Aβ-expressing cells. Finally, we find that DCG aggregate:membrane dissociation is also controlled by cytoskeletal Tau, another key player in AD. We propose a model in which APP plays a central role in DCG compartment maturation, which is disrupted by AD-relevant genetic manipulations, generating phenotypes mirroring defects in early-stage AD and potentially inducing aberrant Rab11-exosome signalling.

204F **TOGARAM1 is required for sperm head-tail connection** Emma Burns[1], Kathleen Holmes[2], Danielle Buglak[2], Brian J. Galletta[2], Nasser M. Rusan[2] [1]Cell and Developmental Biology Center, National Heart, Lung, and Blood Institute, National Institutes of Health, [2]National Heart, Lung, and Blood Institute, National Institutes of Health

Infertility impacts approximately 15% of couples and is due to male infertility in about 50% of these cases. However, most infertility treatment targets females, highlighting a gap in knowledge in male reproduction and sperm development. Sperm are comprised of a head containing the genetic material and a tail acting as a motor. A strong connection between the head and tail is required for successful sperm motility and fertilization. The sperm head and tail are connected by the Head-Tail Coupling Apparatus (HTCA), which is mediated by the basal body, or centriole. Failure of the HTCA leads to partial or complete detachment of the head from its tail, resulting in male infertility. While the importance of this connection is known, the molecular architecture is poorly understood. To discover novel molecular components of the HTCA, we performed a GFP localization screen of candidate proteins and identified the TOG domain protein CG42399, or TOGARAM1, as a novel HTCA protein. TOG domain proteins are important in axoneme organization and cilia length and are linked to ciliopathies, but its function in spermiogenesis is unknown. Utilizing expansion microscopy, we localized TOGARAM1 to the HTCA during *Drosophila* spermiogenesis. Using RNAi, we found that loss of TOGARAM1 led to multiple HTCA phenotypes, including abnormal alignment between the head and tail, in some cases, complete detachment, and aberrant localization of known HTCA proteins, Spag4 and Yuri. This preliminary data suggests that TOGARAM1 is required to maintain a secure attachment at the HTCA. I hypothesize that within the molecular linkage between the centriole and the nucleus, TOGARAM1, through its TOG domain, is involved in regulation of microtubules at the manchette, a cytoskeletal-based structure that facilitates nuclear shaping, indirectly stabilizing the head-tail connection. My current work aims to visualize manchette localization with the loss of TOGARAM1. Similarly, I am investigating TOGARAM1 localization with disruption of the Proximal Centriole Like (PCL) and Nuclear Pore Complexes (NPC) to explore the role of TOGARAM1 within the HTCA linkage. In parallel, I am using turboID as an unbiased biochemical approach to identify interacting proteins of TOGARAM1. This work highlights a novel role of TOGARAM1 in *Drosophila* spermiogenesis and improves our knowledge of the molecular components and mechanisms of the HTCA.

205F **Characterizing nucleolar organization within the germline cells of the *Drosophila* ovary** Anna S Ramsey, Tina L Tootle Biology, University of Iowa

The nucleolus is a membraneless organelle that plays critical roles in cell signaling and has many functions, including ribosome biogenesis, organizing heterochromatin, regulating the cell cycle, and activating the cellular stress response. In amniotes, the nucleolus is organized into three functionally distinct compartments: the fibrillar center (FC), dense fibrillar component (DFC), and granular component (GC). It is currently unclear how many nucleolar compartments are present and how they are organized in the germline cells within the *Drosophila* ovary. To answer this question, we use GFP-tagged protein lines to define the localization of multiple nucleolar proteins within the germline cells of the germaria and Stage 10 follicles. Fibrillarin, a major component of the nucleolus (present in the DFC in amniotes) exhibits relatively uniform staining throughout the nurse cell nucleoli. This uniform staining in the nurse cells is present in both Stage 10 and earlier-stage follicles. Interestingly, Modulo (present in the DFC and the GC in amniotes) and Nucleostemin 1 (present in the GC in amniotes) exhibit holes inside the nucleolar staining pattern in Stage 10B follicles. Fibrillarin is visible within the holes, which suggests the presence of at least two distinct compartments in *Drosophila* nurse cell nucleoli. However, there are no holes in the nucleolar staining of Modulo and Nucleostemin 1 in germ cells within the germarium, indicating organization may differ within the germline cells between stages of follicle development. In addition to the established nucleolar proteins, nuclear actin has recently emerged as a key component of the nucleolus that can affect nucleolar organization. Preliminary data indicates that polymeric nuclear actin localizes within the holes in the nucleolar staining of Modulo and Nucleostemin 1 in Stage 10 follicles. This further supports the idea of the nurse cells having multiple nucleolar compartments, as polymeric nuclear actin is thought to be important for RNA polymerase I activity and rRNA transcription. Ultimately, these studies will advance our understanding the organization of the *Drosophila* nucleolus within the different germline cells and will provide insight into basic nucleolar biology, as well as the similarities and differences between human nucleolar biology and that of *Drosophila*.

206F **Starvation-Induced Lipid Droplets, An Oogenesis Specific Protect Against Starvation Stress** Minhao Chen, Michael Welte Biology, University of Rochester

When female flies are starved, their ovaries are radically remodeled, with cell death and degeneration in the germarium and in Stage 8 follicles. In contrast, Stage 5-7 follicles are much more resistant to starvation-induced degeneration. We found that during the course of starvation the nurse cells (NCs) in these stages transiently accumulate lipid droplets (LDs). LDs are fat storage organelles typically containing triglycerides and sterol esters. Under fed conditions, Stage 5-7 NCs have very few LDs. We observe that LD abundance goes up after 6 hrs of starvation and progressively increases from 12 to 24 hrs. Prolonged starvation leads to disappearance of these LDs (48 hrs) and follicle degeneration (96 hrs). We did not observe similar transient LD accumulation in any other tissue examined, suggesting a follicle specific response. To determine the origin of these starvation-induced lipid droplets (si-LDs), we examined mutants lacking the triglyceride synthesis enzyme DGAT1/Midway. Stage 5-7 NCs accumulate very few LDs upon starvation, suggesting that si-LDs store triglycerides. Intriguingly, in mutants for the triglyceride lipase Brummer/ATGL, Stage 5-7 NCs accumulate LDs even under fed conditions, suggesting that in the fed state LDs are constantly produced and turned over. We will examine whether this lipid flux through LDs supports mitochondrial metabolism, by determining whether various mitochondrial phenotypes are altered in DGAT1 and ATGL mutants. With starvation, the number of LDs increases further in ATGL mutants, suggesting that si-LDs accumulation is not due to loss of ATGL activity. DGAT1 mutant Stage 5-7 follicles develop normally under fed conditions, but they degenerate within 6 hours of starvation, demonstrating a severe reduction in starvation resistance compared to the wild type. Knocking down DGAT1 specifically in the germline also abolishes si-LDs and leads to follicle degeneration upon 24 hrs of starvation. Our observations suggest that si-LDs protect against starvation stress. In the wild, fruit flies endure periods of food shortage, and si-LDs may allow them to retain their reproductive capacity.

Keywords
Lipid droplets, Starvation, Drosophila oogenesis, triglycerides, DGAT1, ATGL

207F **Plexin/Semaphorin Antagonism Orchestrates Collective Cell Migration, Gap Closure and Organ Sculpting** Maik C Bischoff[1], Jenevieve E Norton[2], Mark Peifer[2] [1]Department of Biology, UNC Chapel Hill, [2]UNC Chapel Hill

Cell behavior emerges from the intracellular distribution of properties like protrusion, contractility and adhesion. Thus, **characteristic emergent rules of collective migration** can arise from cell-cell contacts locally tweaking architecture – orchestrating self-regulation during development, wound healing, and cancer progression. **The new *Drosophila* testis-nascent-myotube-system** allows dissection of contact-dependent migration in vivo at high resolution. Here, we describe a process driving gap-closure during migration: **Contact-mesenchymalization** via the axon guidance factor **Plexin A**. This is crucial for testis myotubes to migrate as a continuous sheet, allowing normal sculpting-morphogenesis. We found *plexA* in a large still unpublished RNAseq-guided reverse genetic RNAi screen, among numerous genes affecting collective cell migration and myotube-derived morphogenesis. During migration, cells must stay filopodial and dynamically ECM-tethered near cell-cell contacts to spread while collectively moving. Our data suggest Semaphorin 1B acts as a Plexin A antagonist, fine-tuning activation. Our novel unpublished data reveal a contact-dependent mechanism to maintain sheet-integrity during migration, **driving organ-morphogenesis using a highly conserved pathway**. This is relevant for understanding mesenchymal organ-sculpting and gap-closure in migratory contexts like angiogenesis. Unpublished data also gives first hints at the mechanics of the fascinating **left/right-symmetry-breaking spiral-formation** process after migration, driven by collective mesenchymal alignment and intercalation of myotubes.

208F **Using expansion microscopy to track NP maturation through intracellular compartments of larval *Drosophila melanogaster* brains** Amelia G Mitchell[1], Hardik Bansal[2], Kiel G Ormerod[2] [1]Biology, Middle Tennessee State University, [2]Middle Tennessee State University

Our lab investigates the cellular and molecular mechanisms underlying cellular communication. Our focus is on the largest and most biologically diverse class of signaling molecules, neuropeptides (NPs). There are hundreds of NPs expressed in the human nervous system, most having unique physiological roles. Within neurons, NPs begin as larger precursor proteins called prepropeptides. As this sequence traverses through the endoplasmic reticulum, it gets cleaved into the propeptide and transported into the Golgi apparatus. After further processing in the Golgi apparatus, NPs are ultimately packed into electron dense structures, large dense core vesicles (DCVs). NPs are trafficked down axons to synaptic terminals, or simply released from the soma via canonical exocytosis machinery. This research aims to define a canonical mechanism of NP sorting in motor neurons of Drosophila. Next, we also seek to explore how different NPs are sorted into unique DCVs and define what genetically encoded protein motifs are responsible for governing the transition between phases of sorting. Current experimental approaches are technologically limited by spatial resolution. Even in the Drosophila larval brain, which is relatively simplistic in comparison to the mammalian brain, in vivo sufficient visualization of organelles in the soma is technically not possible using current molecular and microscopy techniques. To combat this, we are using protein expansion microscopy. This allows nanoscale resolution imaging with fluorescence microscopy. Imaging will then reveal where unique NPs localize throughout the sorting pathway and begin to identify genetic mechanisms underlying independent neuropeptide sorting.

209F **Exploring Genetic Connections Between Cardiolipin, CG5755, and Ant2 in *Drosophila melanogaster* Spermatogenesis** Linden Patterson[1], Maggie Woodward[1], Claire Olson[1], Karen G Hales[2] [1]Davidson College, [2]Department of Biology, Davidson College

Dramatic mitochondrial morphogenesis during spermatogenesis makes the *Drosophila* testis a useful context for exploring mitochondrial dynamics. We previously found that *CG5755*, also known as *SLC25A46b*, is required for mitochondrial shaping during late spermatid elongation. Its human ortholog, *SLC25A46*, is associated with several human neurodegenerative conditions. To further functionally characterize *CG5755*, we explored interactions with *Ant2*, whose ortholog, *SLC254A4/5*, has been shown to interact with *SLC254A46*. RNA interference (RNAi) knockdown of *Ant2* revealed its role in male fertility, sperm motility, and testes development. We are investigating if RNAi knockdown of *Ant2* affects the localization of CG5755-GFP. Preliminary data indicate differential localization of CG5755-GFP with *Ant2* knockdown. The paracrystalline material of spermatids was also observed to be an abnormal size relative to their spermatogenesis stage, suggesting that Ant2 may play a role in the coordination of spermatogenesis events. Recent publications suggest that *SLC25A46b* may also interact with cardiolipin, a phospholipid in the inner mitochondrial membrane involved in electron transport chain function, membrane stability, ATP production, and apoptosis. Using RNAi to knockdown the *cardiolipin synthase* (*CLS*) gene, we observed impaired testis development, suggesting cardiolipin's involvement in germ cell proliferation and maintenance. *CLS* knockdown (*CLS*-KD) resulted in disorganized sperm bundles and a significant number of infertile offspring (Olsen, 2023, unpublished). *CLS*-KD had significantly shorter testes and a wider diameter at the basal end compared to wild-type flies, suggesting cardiolipin's involvement in the elongation and individualization stage of sperm bundles during spermatogenesis. *CLS*-KD also led to variable abnormalities in mitochondrial morphology, such as larger nebenkerns in the onion stage of spermatogenesis, emphasizing CLS's importance in mitochondrial dynamics. Similarly, we knocked down the *tafazzin* (TAZ) gene, which encodes a protein involved in cardiolipin remodeling. As with *CLS*-KD, the *TAZ*-KD produced shorter testes, reinforcing the significance of cardiolipin dynamics in testis development and spermatogenesis. While *CLS*-KD and *TAZ*-KD affect testis development and mitochondrial morphology to varying extents, precise mechanisms require further investigation. Overall, these results suggest roles for CG5755, Ant2, CLS, and TAZ in spermatogenesis.

210F **Alterations in Age and Sex Specific Metabolism in a *Drosophila melanogaster* Model of PLA2-VIA Associated Neurodegeneration** Rubaia Tasmin, Anushka Vijay Patil, Surya Jyoti Banerjee Biological Science, Texas Tech University

PLA2G6-Associated Neurodegeneration (PLAN) is a rare, progressive neurodegenerative disorder caused by mutations in the PLA2G6 gene, which encodes calcium-independent phospholipase A2 (iPLA2-VIA). These mutations drive neurodegeneration through disruptions in phospholipid metabolism and mitochondrial function. The *Drosophila melanogaster* model of PLAN carrying loss-of-function mutations in the iPLA2-VIA gene, exhibits age-dependent locomotor defects, shortened lifespan, and female-specific fertility impairments. Given the role of iPLA2-VIA in mitochondrial function, we hypothesized that these flies would present age-dependent, sex-specific metabolic abnormalities.

To investigate, we used mass spectrometry to analyze small molecules and lipids in young and old iPLA2-VIA^{LOF} mutant and control flies. We identified 195 small metabolites and 379 lipids that met quality standards. In aged male iPLA2-VIA^{LOF} mutants, we detected significant dysregulation in 27 upregulated and 55 downregulated metabolites, along with 3 upregulated and 303 downregulated lipids. In aged female iPLA2-VIA^{LOF} mutants, 46 metabolites were upregulated and 36 downregulated, while lipid profiling showed 130 upregulated and 102 downregulated lipids. Principal component analysis (PCA) revealed that the metabolic profile of young iPLA2-VIA^{LOF} mutant males closely resembled controls, but aged flies demonstrated partial separation. In contrast, female mutants displayed distinct metabolic profile separation, with young mutant females resembling aged controls, indicative of premature aging.

Pathway analysis via Ingenuity Pathway Analysis (IPA) software highlighted disruptions in glycolysis, the TCA cycle, and lipid metabolism. Notably, ATP assays with the ovaries of female flies revealed that ATP levels in the ovaries of iPLA2-VIA^{LOF} mutant females were lower than those in control females, pointing to mitochondrial dysfunction's role in fertility defects. In addition, reactive oxygen species (ROS) levels were found to be elevated in iPLA2-VIA^{LOF} mutant flies, suggesting increased oxidative stress that exacerbates mitochondrial damage and energy deficits. A reduction in mitochondrial DNA (mtDNA) levels was also observed in 7-day-old mutant males, potentially contributing to reduced lifespan, and locomotor impairments seen in these mutants.

These findings could help us understand how mitochondrial problems, fat metabolism, and oxidative stress contribute to neurodegeneration in PLAN.

211F **Investigating the Effects of Microtubule Acetylation on Neuronal Development and Behavior in Drosophila Melanogaster** Sophia Trujillo[1], Chloe Welch[1,2], Helen Than[3], Ethan Schauer[3], Jill Wildonger[1,2,3] [1]School of Biological Sciences, Cell & Developmental Biology, University of California, San Diego, [2]Biological Sciences Graduate Program, School of Biological Sciences, University of California, San Diego, [3]School of Medicine, Pediatrics, University of California, San Diego

Microtubules are a fundamental part of the cytoskeleton that shapes cell structure, organization, intracellular transport, and development. Microtubules consist of alpha- and beta-tubulin dimers, which attach together to form a tubular structure. The dynamic nature of microtubules directly affects the structure of developing neurons and their ability to function properly. The stability and dynamics of microtubules can be regulated by post-translational modifications, such as acetylation. Multiple conserved lysine (K) residues in alpha-tubulin are acetylated, but the significance of acetylation at many of these sites is still unknown. We mutated three of these sites in alpha-tubulin (K326, K370, K401) to prevent their acetylation and assayed for effects on microtubule stability, neuromuscular junction development, and larval crawling. We found that mutagenesis of K326 decreases microtubule stability, reduces the number of boutons at the neuromuscular junction, and disrupts larval locomotion. In contrast, mutagenesis of K370 or K401 did not affect microtubule stability, although neuromuscular junction development was perturbed. Our data suggest that K326, and potentially its acetylation, plays a role in regulating the stability and dynamics of the microtubule cytoskeleton in a way that impacts the development and function of the neuromuscular junction.

212F **An expanding role of Protein kinase N (Pkn) as a Rho1 effector in *Drosophila melanogaster*.** Molly McGuire[1], Lia Quatro[2], Georgette Sass[2] [1]Biochemistry, Grand Valley State University, [2]Biology, Grand Valley State University

The ability of Rho GTPases to mediate transduction of regulatory signals and modulate cellular activities depends on interaction with downstream effectors. Rho1 effectors in *Drosophila melanogaster* play roles in nucleation and elongation of actin filaments (diaphanous), as well as promotion of actin filament stabilization (LIM kinase) and actomyosin contractility (Rho kinase), for example. Analysis of *Protein kinase N* (*pkn*) mutants identified Pkn as a Rho1 effector with associated defects in embryonic dorsal closure and a role in the negative regulation of actin-myosin contraction during nurse cell dumping. These aspects of Pkn as a Rho1 effector are in keeping with Rho GTPase family member's function as key actin cytoskeleton regulators. We have characterized the *delorean* mutant allele of *pkn* (*pkn^{dln}*) which exhibits pairing-dependent reduction in levels of *pkn* expression. A reduction of maternally contributed Pkn protein from *pkn^{dln}* homozygous females reveals a range of embryonic defects. We focused on the earliest embryonic defects where loss of nuclei from the embryo periphery prior to cellularization was evident. Nuclear loss was also accompanied by centrosome loss as visualized by the absence of a-tubulin staining (DSHB 4A1). A GFP-tagged form of Pkn was used to examine the endogenous localization of the Pkn protein to better understand its role in early embryonic actin-cytoskeleton dynamics. We examined pre-cellularization stages (nuclear cycles 1-13) as well as embryos undergoing cellularization (nuclear cycle 14). Detection of Pkn-GFP in syncytial blastoderm as nuclei migrate outward to the embryo cortex was clearly seen in nuclear cycle 8 and an association with centrosomes was detected as early as nuclear cycle 10. Furthermore, Pkn-GFP was found to be localized to ingressing plasma membrane furrows and specifically localized at the basal tip of the cleavage furrow during cellularization (nuclear cycle 14). A maternal loss-of-function phenotype was created using a *nos*-GAL4 driver and either CRISPR-Cas9 mediated knockout or deGradFP knockdown of Pkn-GFP. We were able to recapitulate embryonic phenotypes first seen with homozygous *pkn^{dln}* mothers such as the loss of cortical nuclei and centrosomal staining. To further characterize Pkn localization during early embryogenesis, the integrity of the actin cytoskeleton was visualized in homozygous *pkn^{dln}* individuals containing the moesin actin-binding domain fused to Scarlet. These individuals exhibited enhancement of the mutant wing phenotype of *pkn^{dln}* homozygous adults. Moreover, this enhanced phenotype was also seen when an EGFP-tagged actin binding domain from human utrophin or a GFP-tagged moesin gene was present in the *pkn^{dln}* homozygous background. We discuss how these unexpected results in conjunction with Pkn expression patterns in early embryogenesis contribute to our understanding of Pkn as a Rho1 effector.

213S **The MAST Kinase Drop out controls polarized membrane growth through a Rab11/Nuf - dependent recycling endosome pathway by phosphorylation of Dynein-light intermediate chain** Benedikt Drebes[1], Sabine Pautz[2], Hannah C Sonnenberg[3], Alistair J Langlands[3], Katja Kapp[1], Friedrich W Herberg[2], Hans-Arno J. Müller[4] [1]Developmental Genetics, University of Kassel, [2]Biochemistry, University of Kassel, [3]Cell and Developmental Biology, University of Dundee, [4]Developmental Genetics, Universität Kassel

Early embryogenesis is associated with a substantial growth of plasma membrane surface area. In *Drosophila* embryos, polarized membrane growth during cellularization is fueled by various sources including vesicles derived from the recycling endosome. Embryos derived from mothers homozygous for loss-of-function alleles of *drop out* (*dop*) exhibit severely reduced membrane growth during cellularization. *dop* encodes the single *Drosophila* homolog of Microtubule associated Serine/Threonine (MAST) kinases, a highly conserved branch of the AGC Kinase family, which are associated with many human diseases. In a quantitative phospho-proteomics experiment we identified the Dynein light intermediate chain (Dlic) as a potential substrate of Dop. Furthermore, an *in vitro* phosphorylation study revealed the evolutionary conserved serine residue 401 in the carboxyterminal tail of Dlic as a phosphorylation target site of Dop. Overexpression of phospho-mimetic variants of Dlic Ser 401 resulted in the suppression of several phenotypes of *dop*embryos, including a significant rescue of embryonic lethality and restoration of membrane growth. Membrane growth in early cellularization requires transport from the recycling endosome involving the small GTPase Rab11 and the Dynein adaptor Nuclear fallout (Nuf). Overexpression of Rab11 or Nuf in *dop* mutant embryos suppressed the membrane growth defect and the lethality of *dop* mutants. To investigate the extent to which a negative charge on Ser 401 of Dlic influences its interaction with the Dynein adaptor Nuf, we performed *in vitro* binding assays between Dlic and Nuf. We found that a negative charge reduced the interaction between an intrinsically disordered region within the carboxyterminal tail of Dlic and the central coiled-coil domain of Nuf. These data support the model that phosphorylation of Dlic Ser 401 by Dop weakens the interaction between Nuf and Dlic to promote Rab11/Nuf-dependent transport through the recycling endosome.

214S **Investigating the regulation of v-ATPase-mediated autolysosomal acidification** Amanda Scharenbrock, Thomas P Neufeld Genetics, Cell Biology, & Development, University of Minnesota Twin Cities

Macroautophagy, henceforth referred to as autophagy, is a process by which cellular components are sequestered into a double-membraned autophagosome which fuses with the lysosome to create an autolysosome, the ultimate site of degradation of the sequestered cellular components. An important component of degradation is lysosomal acidification via the Vacuolar H^+-ATPase (v-ATPase), located on the lysosomal membrane. There are other components located on the lysosomal membrane that may also contribute to acidification, but these roles have yet to be fully defined. One protein of interest is mechanistic Target of Rapamycin (mTOR), a nutrient sensing protein kinase. mTOR is a key regulator of autophagy and resides in the same complex as v-ATPase on the lysosomal membrane. In *Drosophila* larval fat body, inhibition of mTOR results in lysosomal acidification in a well-fed state when acidification is not typically seen, whereas mTOR activation blocks acidification under starvation conditions. Overall, this supports an important role for mTOR in lysosomal acidification. One theory of mTOR's involvement in acidification is that mTOR regulates assembly of v-ATPase at the lysosomal membrane through inhibitory mechanisms. In mammalian cell culture, there is evidence of both mTOR-independent and dependent v-ATPase assembly. Furthermore, uncharacterized ion channels in the lysosomal membrane may contribute to acidification, potentially through interactions with v-ATPase or mTOR. mTOR also regulates the formation of autophagosomes, whose fusion with the lysosome may play a role in its acidification. In the fat body, mutation or depletion of Autophagy-related (Atg) proteins disrupts autophagosome formation and leads to a lack of lysosomal acidification, indicating that acidification is dependent on autophagosome formation, autophagosome-lysosome fusion, and/or non-canonical roles of Atg proteins. Using a newly developed genetically encoded lysosomal pH sensor, we will describe our investigations of the mechanisms of mTOR regulation of v-ATPase and fusion-mediated lysosomal acidification.

215S **AAA+ ATPase paralogs Nmd and CG4701 are Required for Mitochondrial Shaping and Peroxisomal Maintenance in *Drosophila melanogaster* Spermatogenesis.** Amelia Roselli[1], Willow Pagon[2], Ummer Qureshi[2], Elizabeth Young[2], Vickie Williams[2], Karen G Hales[1] [1]Biology, Davidson College, [2]Davidson College

Drosophila paralogs *nmd* and *CG4701*, orthologs of human *ATAD1* and yeast *MSP1*, localize to the outer mitochondrial membrane and peroxisomes. Previous published studies of this protein family in immortalized human cell lines and yeast show the gene products to be integral membrane protein extractases on the mitochondrial membrane. We identified a recessive male sterile *nmd* mutant with disrupted mitochondrial aggregation, $nmd^{p(ry)4}$, with a P element inserted in a region of the gene only included in one of the three nmd transcripts. This suggests that *nmd* may have a testis specific transcript, which we investigated using RT qPCR. Our data suggest that there is a difference in tissue specific expression levels between the three transcripts in the testis and other organ systems. Research in yeast suggests a relationship between Nmd and peroxisomal maintenance, which we investigated in Drosophila spermatogenesis. We observed that loss of Nmd resulted in diffuse peroxin signals (SKL-GFP), as opposed to distinct puncta in wildtype, which indicates that peroxins are not localizing to peroxisomes in Nmd knock down. We further expressed PMP34-Cerulean and identified a loss of peroxisomal membranes when Nmd was knocked down, suggesting that Nmd is required for peroxisomes to exist in spermatogenesis, either by protecting peroxisomes from peroxiphagy or by supporting peroxisome biogenesis. To this end, we are exploring how peroxin signals and CG4701 localization changes when peroxisomal biogenesis is disrupted. Additionally, research suggests that MSP1 extracts mislocated tail anchored proteins from the outer mitochondrial membrane, so we are disrupting the guided entry for tail anchored proteins (GET) pathway to explore how Nmd localization changes.

216S **RhoA and its effectors uniquely modulate border cell cluster texture and mechanics** Emily G Gemmill[1], Joseph P Campanale[2], Allison M Gabbert[3], Noah P Mitchell[4], Denise J Montell[5] [1]Interdisciplinary Program in Quantitative Biosciences, University of California, Santa Barbara, [2]School of Life Sciences, University of Nevada, Las Vegas, [3]Division of Cardiovascular Medicine and Division of Hemostasis and Thrombosis, Beth Israel Deaconess Medical Center and Harvard Medical School, [4]Department of Molecular Genetics and Cell Biology, University of Chicago, [5]Molecular, Cellular, and Developmental Biology, University of California, Santa Barbara

Collective cell migration is critical for processes such as wound healing and development. Border cell clusters are an *in vivo* model from developing *Drosophila* ovaries. In this system, 5-7 cells detach from the anterior epithelium, squeeze between giant nurse cells as they migrate, and then reattach at the oocyte. Border cell clusters, with a single leader, demonstrate cell-on-cell migration and have easily quantifiable migration defects. To migrate successfully, border cells balance cortical stiffness and deformability, two inverse properties primarily controlled by the cytoskeleton. Rho, a GTPase, regulates the actin cytoskeleton and is critical for migration. Rho plays this role through its effectors, such as septin, an understudied cytoskeletal element that takes on structures such as rings, gauzes, and filaments. Previously, we found that septin is necessary for migration. Septin is a highly conserved protein that modulates the surface texture and shape of the border cell cluster. We propose that these properties serve as proxies for cortical stiffness and deformability, respectively. This raises the key question: how do Rho and its effectors modulate surface texture? To answer this question, we use high-resolution Airyscan imaging and create a 3D mesh of the cluster using ImSAnE. The texture of this mesh is quantified using spectral decomposition. This work shows how Rho and its effectors Rho kinase, myosin, Moesin, and diaphanous also affect the cluster texture and shape, each with distinct phenotypes hinting at their role in cluster and migration mechanics.

217S **Comparative Analysis of Clinical Variants in the Functional Domains of DNM1L Reveals a Spectrum of Peroxisomal and Mitochondrial Alterations in *Drosophila* Larval Muscle** Saurabh Srivastav[1], Neelanjana Roy[1], Jonathan Andrews[1], Sharayu Jangam[1], Aanya Subramaniam[2], Shinya Yamamoto[1], Michael Wangler[1] [1]Molecular & Human Genetics, Baylor College of Medicine, [2]Baylor College of Medicine

DNM1L encodes dynamin-related protein 1 (DRP1) an 80-kDa GTPase in the dynamin super-family which is essential for mitochondrial dynamics. Dysfunction in DNM1L has been linked to mitochondrial and neurodegenerative disorders, including Alzheimer's, Parkinson's, and Huntington's disease. DNM1L protein has 3 distinct domains, including a GTPase domain (GD), a middle domain (MD) and the GTPase effector domain (GED). To date, different domain specific variants have been identified in the DNM1L gene among patients who exhibit a range of neurological symptoms including severe hypotonia, cerebellar atrophy, encephalopathy, isolated optic atrophy, profound intellectual disabilities, and gross motor delay. So far, functional studies of variants in cell lines and patient's fibroblast have depicted anomalies in mitochondrial or/and peroxisomes linked to clinical spectrum of DNM1L related disorders. Here we detail the functional studies in fly drp1 null or sensitized background, involving overexpressing the novel GED variant (L700del) in *Drosophila* and compare the phenotypes this variant produces in our model system with GD (A192E) and MD (R403C) fly models produced in our lab. We find that our *Drosophila* model displays locomotor dysfunction and an accumulation of elongated tubular peroxisomes along with swollen enlarged mitochondria when overexpressing the GED variant (L700del) and middle domain variant (R403C). These effects are comparable to that of middle domain missense alleles observed in pediatric subjects with defective mitochondrial and peroxisomal fission 1 (EMPF1, MIM #614388). The results clearly indicate that our *Drosophila* model can produce EMPF1 phenotypes and could be potentially utilized as model for the development of therapeutics. Further, the current study enhances our understanding of the natural progression and mechanism of an increasing number of DNM1L-related disorders.

219S **Snail-dependent downregulation of junctional Bazooka during epithelial-mesenchymal transition** Yasong Pang, Mo Weng, Marissa Teng University of Nevada, Las Vegas

Epithelial-mesenchymal transition (EMT) converts connected epithelial cells to detached mesenchymal cells by downregulating cell adhesion and apicobasal polarity. EMT is essential for development and homeostasis, and when misregulated promotes tumorigenesis. EMT used to be considered a binary process but the accumulation of evidence has pointed to a model where cell states reversibly transition along an epithelial-mesenchymal spectrum with intermediate states. However, the molecular mechanisms of intermediate states are poorly understood. Using Drosophila embryonic mesoderm, our previous studies suggest a mechanism for intermediate states: the conserved master EMT transcription factor Snail disassembles adherens junctions while preserving junctional proteins through downregulating polarity protein Bazooka/Par-3 (Baz). Here, we show that Snail does not change Baz protein levels but reduces Baz cluster formation at junctional cortex which is essential for Baz's function in assembling and maintaining adherens junctions. We found that this Baz downregulation function of Snail depends on its nuclear localization and likely its transcription role since the non-nuclear mutant Snail fails to downregulate junctional Baz. We tracked individual Baz clusters at the junctional cortex and found them to be highly dynamic, undergoing fusion, splitting, growth and shrinkage. Before Snail takes effect, there is an upward mobility and an upper limit in cluster size and intensity for the junctional Baz clusters. The probability to grow bigger or fuse is higher in small clusters but the probability to shrink does not depend on size. Overall, smaller clusters grow bigger but once they reach the limit, they tend to decrease in size and intensity. Snail expression reduces Baz clusters in intensity and density through suppressing cluster growth and fusion while promoting shrinkage and death. In response to Snail expression, the probability of cluster growing or undergoing fusion gradually decreases while that of shrink and death decrease. By contrast, splitting is not regulated by Snail expression. Our results show that Snail promotes reversible junction loss by limiting cortical Baz clustering.

220S **LINC(ing) Nucleoporins in *Drosophila* myogenesis** Arun Kumar[1], Richard Cripps[1], Kumar Vishal[2], Maya Capelson[3] [1]Biology, San Diego State University, [2]San Jose State University, [3]San Diego State University

All genetic material in eukaryotic cells is sequestered within a double-layered nuclear envelop, which comprises an inner and outer nuclear membrane. Several transmembrane proteins are located on the nuclear envelope, but only two integral protein complexes fully span the nuclear envelope, connecting the nucleus to the cytoplasm. Embedded in the nuclear membrane are the massive Nuclear Pore Complex (NPC) composed of almost 30 different types of protein called nucleoporins (Nups) which facilitate molecular exchange between the nucleus and the cytoplasm. Meanwhile, LINC ((Linker of Nucleoskeleton and Cytoskeleton) complexes physically connect the nucleoskeleton to the cytoskeleton, playing a crucial role in nuclear positioning and mechanotransduction. However, their specific role in muscle function remains poorly understood.

The assembly of NPCs and their even distribution throughout the nuclear envelop is dependent on the component of LINC complex. Dysregulation of this complex is associated with muscle-related disorders, including myofiber atrophy. To identify novel genes critical for development of body wall muscle function, a genetic RNAi screen targeting known Nups was conducted. This screen identifies several new regulators of muscle, including Nup155, an inner ring core protein involved in transport and chromatin function. To study the tissue specific function of this gene, Nup155 was specifically knocked down using RNA interference in body wall muscle. Importantly, muscle specific knockdown of this gene in flies induces larval lethality. Perturbation of LINC complex after knockdown of Nup155 in larval muscle led to significant decrease in the amount of *Klar* (KASH protein in the outer nuclear membrane), indicated an altered nuclear pattern and mispositioned in the muscle fiber. These data suggest that nuclear associated LINC complex might contribute to lethality in larval muscle.

221S **Dissecting the Spir/Capu interactome and the role it plays in the regulation of the actin mesh** Carolyn Wu, Hee Jong Kim, Merin Rixen, James Wohlschlegel, Margot Quinlan UCLA

The actin cytoskeleton is essential for the development of a viable egg. During *Drosophila* oogenesis, an actin mesh appears throughout mid-oogenesis but disappears by late-oogenesis. The timely regulation of this mesh is crucial for properly localizing determinants that establish cell polarity and define the future patterning of the embryo. The mechanism and factors behind mesh maintenance and removal remain elusive. Here, we used proximity labeling and co-immunoprecipitation to map interactors of Spire (Spir) and Cappuccino (Capu), two known actin nucleators that directly interact with each other and are essential to build the mesh in *Drosophila* oocytes. Understanding the Spir/Capu pathway will allow deeper insight into the role of the actin cytoskeleton during oogenesis and potentially translate to mammalian systems where the same set of actin nucleators are used to build a similar actin network.

222S **Macrophage invasion is affected by mechanics of surrounding cells through positioning of divisions** Maria Akhmanova, Xiaoqun Catherine Zhang Cell Biology Program, Memorial Sloan Kettering Cancer Center

Tissue-resident macrophages arrive at all the organs during early embryogenesis and their dissemination relies on their ability to penetrate (*invade*) and move through confined environments, which provide not only a substrate but also resistance to forward movement [1,2]. The mechanisms which allow cells to overcome these challenges are largely unknown. Previously, we have demonstrated that in *Drosophila* embryos, dividing ectodermal cells are required for macrophage invasion because they disassemble cell-cell attachments formed by integrin-mediated focal adhesions next to mesodermal cells, allowing macrophages to move their nuclei ahead and invade between two immediately adjacent tissues. Invasion efficiency depends on division frequency, but reduction of adhesion strength allows macrophage entry independently of division [3]. Moreover, recent study in this *in vivo* model shows that the increase in tension of the ectodermal cells in *egr* mutant impedes invasion of the macrophages [4]. Here we aimed to understand how mechanistically does the elevated tension resist invasion. We found that increased tension alters the position of the cell division, forcing cells to move to apical side of the ectodermal layer when they divide. This movement leads to decrease in division frequency at the sites of macrophage entry and prevent invasion. Thus, our results suggest that mechanical changes in tissue cells influence invasion efficiency by affecting division location. Our findings will help to uncover the principles of interplay between invading cells and surrounding tissues in physiological and disease contexts.

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223S **Cofilin modulates survival of heat stressed embryos** Faizan Rashid[1], Natalie Biel[1,2], Subhashis Natua[3], Thu Vu Phuc Nguyen[4,5], Ido Golding[4,5], Auinash Kalsotra[3], Anna Marie Sokac[1] [1]Cell and Developmental Biology, University of Illinois at Urbana Champaign, [2]Integrative Molecular and Biomedical Sciences, Baylor College of Medicine, [3]Biochemistry, University of Illinois at Urbana Champaign, [4]Physics, University of Illinois at Urbana Champaign, [5]Microbiology, University of Illinois at Urbana Champaign

Cofilin regulates polymerization of the actin cytoskeleton. Cofilin also acts during cellular stress response to promote survival or death depending on context. Yet, it is unclear how Cofilin interacts with stress response. We find that Cofilin induces an Actin Stress Response (ASR) in Drosophila embryos exposed to acute or chronic elevated temperature (32oC). As part of the ASR, Cofilin destabilizes cytoplasmic actin filaments, causing mild morphogenesis defects, and drives assembly of nuclear actin rods. These actin perturbations of the ASR coincide with reduced embryo survival. Cofilin knockdown restores survival, but not by mitigating these actin perturbations of the ASR. Instead, Cofilin knockdown primes embryos, setting them on a distinct trajectory that blunts multiple stress responses, including ER stress response, and buffers perturbation of developmental gene expression. In addition, Cofilin knockdown fully rescues the survival of heat stressed embryos. We conclude that Cofilin modulates stress response pathways beyond the ASR and that Cofilin is a critical determinant of survival when embryos encounter environmental stress. In future experiments, we will explore if Cofilin's regulation of stress response pathways is direct or somehow occurs indirectly via actin.

224S **Collective filopodia dynamics during sensory bristle pattern formation in Drosophila** Sushmita Kundu Biology, Clarkson University

During development, multicellular organisms exhibit many tissues that self-organize. The array of sensory bristles on the notum of Drosophila is a model system for self-organized tissue patterning. During the formation of this bristle pattern, actin-rich signaling filopodia extend from the basal surface of all epithelial cells to support long-range Notch signaling and support spacing between bristles. Signaling filopodia, or cytonemes, are long, dynamic cellular protrusions facilitating cell-cell communication. However, how they interact with each other is not well understood. We observed collective filopodia structures during patterning and hypothesized that they contribute to the development of the bristle pattern. To quantify their movement, we used particle image velocimetry analysis and manual analysis to measure their collective movements during the patterning of Drosophila notum at the tissue level, in vivo. We found that collective structures are dense meshworks of intertwined signaling filopodia and measured several parameters including magnitude of structure movement, structure size, direction of movement, and rate of movement. Experiments using dominant negative mutants of the non-muscle myosin II regulatory light chain show an increased movement of structures relative to controls. To track both the movement of a subset of filopodia and collective filopodia movements, we tracked the interactions of individual cells in collective structures. To investigate cell adhesion in filopodia structures, we visualized zyxin and E-cadherin in these structures, in parallel with an RNAi- approach to determine which adhesions contribute to the formation of collective filopodia structures. Altogether these results indicate that interactions between signaling filopodia are dynamic and complex during patterning.

225S **SLMO transfers phosphatidylserine between the outer and inner mitochondrial membrane in *Drosophila*** Siwen Zhao, Xuguang Jiang, Ning Li, Tao Wang National institute of Biological Sciences, Beijing

Phospholipids are critical building blocks of mitochondria, and proper mitochondrial function and architecture rely on phospholipids that are primarily transported from the endoplasmic reticulum (ER). Here, we show that mitochondrial form and function rely on synthesis of phosphatidylserine (PS) in the ER through phosphatidylserine synthase (PSS), trafficking of PS from ER to mitochondria (and within mitochondria), and the conversion of PS to phosphatidylethanolamine (PE) by phosphatidylserine decarboxylase (PISD) in the inner mitochondrial membrane (IMM). Using a forward genetic screen in *Drosophila*, we found that Slowmo (SLMO) specifically transfers PS from the outer mitochondrial membrane (OMM) to the IMM within the inner boundary membrane (IBM) domain. Thus, SLMO is required for shaping mitochondrial morphology, but its putative conserved binding partner, dTRIAP, is not. Importantly, SLMO's role in maintaining mitochondrial morphology is conserved in humans via the SLMO2 protein and is independent of mitochondrial dynamics. Our results highlight the importance of a conserved PSS-SLMO-PISD pathway in maintaining the structure and function of mitochondria.

226S **Genetic dissection of motor proteins mediating dense core vesicle axonal trafficking in *Drosophila*** Aidan Dermady[1,2], Jun Yin[1], Caixia Long[1], Hsueh-Ling Chen[1], Quan Yuan[1] [1]National Institute of Neurological Disorders and Stroke, [2]Brown University

Neuropeptides are known to act as modulators of development and a variety of behaviors, but the mechanisms and events controlling their release are not well understood. One such key event is the bidirectional transport of neuropeptide-containing dense core vesicles (DCVs) along the axon tract. While many studies of DCV transport in *Drosophila* have been conducted in motor neurons, here we use the pigment dispersing factor (PDF)-releasing peptidergic lateral ventral neurons (LNvs) as a model system to study the regulation of axonal DCV transport. Using fixed staining, live imaging, and behavioral analysis, we found that, as shown previously in other peptidergic neurons, Unc-104 and Dhc64c, are required for anterograde and retrograde transport of PDF, respectively. However, we also found additional motors that may play a role in DCV transport in the LNvs, such as the kinesins Klp68D and Khc-73, and most interestingly, the axonemal dynein subunits CG9313 and Dhc93AB. While axonemal dyneins typically do not contribute to cytoplasmic transport, we hypothesize that these subunits may nevertheless act as regulators of axonal DCV transport in the LNvs. Additionally, live imaging in third instar larval LNvs reveals potential circadian regulation of axonal DCV transport, which may play a role in the oscillation of synaptic PDF levels throughout the day. These findings may further our knowledge of mechanisms regulating DCV axonal transport in the *Drosophila* LNvs.

227S **Drosophila Clu ribonucleoprotein particle dynamics rely on the availability of functional protein and polysome stability** Hye Jin Hwang[1,2], Kelsey M Sheard[3], Rachel Cox[4] [1]Biochemistry and Molecular Biology, Uniformed Services University, [2]Henry M. Jackson Foundation for the Advancement of Military Medicine, [3]Meso Scale Diagnostics LLC, [4]Uniformed Services University

Ribonucleoprotein (RNP) particles are important for posttranscriptional regulation of mRNAs. *Drosophila* Clu is a conserved multi-domain ribonucleoprotein essential for mitochondrial function that forms dynamic RNP particles within the cytoplasm. Unlike stress granules and Processing bodies, Clu particles disassemble under nutritional or oxidative stress. However, it is unclear how disrupting protein synthesis affects Clu particle dynamics, especially since Clu binds mRNA and ribosomes. Here, we capitalize on *ex vivo* and *in vivo* imaging of *Drosophila* female germ cells to determine what domains of Clu are necessary for Clu particle assembly and how manipulating translation affects particle dynamics. Using domain deletion analysis, we identified three domains in Clu, essential for particle assembly. We also demonstrated that overexpressing functional Clu disassembled particles, which suggests phase separation. In addition, we inhibited translation using cycloheximide and puromycin. In contrast to Processing bodies, cycloheximide treatment did not disassemble Clu particles yet puromycin treatment did. Surprisingly, cycloheximide stabilized particles under oxidative and nutritional stress with normal mitochondrial distribution. Considering that the presence of Clu particles correlates with proper mitochondrial distribution in germ cells, this supports Clu particles also govern mitochondrial localization. These findings demonstrate that Clu RNP particles display novel dynamics in response to its functional protein level and altered ribosome activity and support a model where they function as translation hubs whose assembly heavily depends on the dynamic availability of polysomes, giving insight into how these unique RNP particles regulate mitochondrial biology in *Drosophila*.

228S **Investigating the tissue-specific impact of EMC4 knockdown on fertility and lifespan in *D. melanogaster*** Salma Abdelkhalek, Otoha Tatami, Rebecca Delventhal Biology/Neuroscience, Lake Forest College

The endoplasmic reticulum (ER) membrane protein complex (EMC) plays a vital role in the proper folding, insertion, and trafficking of membrane proteins. In other work from our lab, we found that EMC4 knockdown in glial cells of *Drosophila melanogaster* leads to significantly reduced lifespan and developmental delays. In addition to the nervous system, another system that relies on signaling between different cell types is the regulation of fertility and reproduction. We hypothesize that the EMC may play a role in this inter-organ signaling through its involvement in the biogenesis of membrane proteins. To investigate this, we used the GAL4-UAS system to knockdown EMC4 in various tissues known to signal to the ovaries: hemocytes, the fat body, and insulin-producing neurons. Fertility assays were conducted in young (4-5 day old) and aged (21-23 day old) females, measuring eggs laid (fecundity) and eggs hatched (fertility). We discovered distinct tissue-specific effects of EMC4 knockdown on fertility and fecundity. Knockdown in the fat body showed the most robust impact on fertility and fecundity compared to other tissues; the effect of knockdown in some tissues was age-dependent, whereas others were observed in both young and old females. These findings suggest a potential tissue-specific requirement for EMC4 in regulating reproductive capacity. Given the well-known tradeoff between life history traits of reproduction and lifespan, we wondered whether EMC4 knockdown in these tissues would also influence adult lifespan. Contrary to expectations, lifespan data showed that knockdowns that decreased fertility did not correspond with increases in lifespan, indicating that the predicted tradeoff was not observed in all tissues. These results hint at a tissue-specific role of EMC4 in aging, independent of reproduction. Future work will examine how EMC4 knockdown in signaling tissues affects key stages of egg development, including follicle maturation. These studies aim to provide a better understanding of the role of EMC4 in tissue-specific protein biogenesis and inter-organ communication, shedding light on its importance in maintaining reproductive and overall organismal health.

229T **Mutant *C.3.3* identified from a conditional Flp/FRT EMS screen harbors lethal mutations in *Rpe* and *Nup75*** Mariana Gonzalez[1], Kayla Bieser[2], Cory Evans[3], Jacob D Kagey[4] [1]University of Detroit Mercy, [2]Biology, Nevada State University, [3]Biology, Loyola Marymount University, [4]Biology, University of Detroit Mercy

A conditional Flp/FRT EMS mutagenesis screen was conducted in the *D. melanogaster* adult mosaic eye to look for regulators of the cell cycle, cell division, and tissue development. The starting chromosome for this screen harbored an allele of *Dark82* that blocks the canonical apoptosis pathway in the mosaic mutant tissue. The screen identified secondary mutations that disrupted the cell growth and developmental patterning in the adult eye. One mutant from the screen, *C.3.3,* was characterized by having a reduction in the ratio of mutant tissue to wild type tissue in the mosaic eye. This mutant was genetically mapped by undergraduates in the Fly-CURE consortium at Loyola Marymount University and Nevada State University. This group identified two distinct lethal mutations on the right side of chromosome 2. The lethal mutations were further mapped to specific genes, *Rpe* and *Nup75*. Here we aim to identify if one of the mutations (or both) is responsible for the *C.3.3* mosaic eye phenotype. Using recombination, we found that *Rpe* and *Dark82*, had a similar phenotype to *C.3.3* overall (*Dark82*, *Nup75*, and *Rpe*) suggesting that *Rpe* is driving the conditional mosaic eye phenotype. Currently, we are working on the generation of the *Nup75*, *Dark82*mosaic eye to determine if both mutants drive the same phenotype. Overall, these data will lead to a better understanding of the interplay between multiple mutants in the developing *Drosophila* eye.

230T **Investigating the role of 2-hydroxyglutarate in *Rp/+ Minute* cell physiology and cell competition.** Alex Mastrogiannopoulos[1], Eugenia Piddini[2] [1]School of Cellular and Molecular Medicine, University of Bristol, [2]University of Bristol

Mutations in ribosome protein (*Rp*) encoding genes, known as *Minutes,* and in ribosome biogenesis factors result in debilitating diseases known as ribosomopathies and contribute to complex genetic diseases like cancer. Cells heterozygous mutant for *Rp* genes (*Rp*/+) also get eliminated by cell competition when mixed with wild-type cells, which is thought to act as a quality control mechanism to remove unfit cells. In a recent genetic screen of factors that affect cell competition, we found that metabolic enzyme levels contribute to loser elimination in cell competition. Following this observation, we performed metabolomics in *Rps3* +/- and wild-type discs and identified various metabolites differentially abundant in *Rps3* cells. Notably, these include higher 2-hydroxyglutarate and lower alpha ketoglutarate levels in *Rp*/+ cells compared to wild-type. We altered the expression levels of *L2hgdh*, an enzyme that catalyses the conversion of 2-hydroxyglutarate to alpha ketoglutarate, in losers, and observed that knockdown of *L2hgdh* increases loser elimination while overexpression reduces loser death to below control competition levels. One of the key known functions of 2-hydroxyglutarate is to inhibit alpha ketoglutarate catalysing dioxegynases, including histone demethylases. To examine the function of 2-hydroxyglutarate in *Minutes*, we fed 2-hydroxyglutarate to *Rp*/+ larvae and observed elevated Histone 3 Lysine 9 trimethylation (H3K9me3), indicating that 2-hydroxyglutarate represses alpha ketoglutarate driven histone demethylase activity in *Rp*/+ cells. Taken together, our observations propose a model where 2-hydroxyglutarate metabolism alters the chromatin state of *Rp*/+ cells, thus affecting their survival during cell competition.

231T **Sex-dimorphic tumor growth is regulated by tumor microenvironmental and systemic signals** Xianfeng Wang, Hongcun Bao, Yi-Chun Huang, Anindita Barua, Chun-Ming Lai, Jie Sun, Youfang Zhou, Fei Cong, Shangyu Gong, Chih-Hsuan Chang, Wu-Min Deng Tulane University School of Medicine

Tumor growth and progression involve coordinated regulation by internal, microenvironmental and systemic signals, and often display conspicuous sexual dimorphism. The mechanisms governing the integration and coordination of these signals, along with their sex-based differences, remain largely unknown. Using a *Drosophila* tumor model originating from non-reproductive tissue, we show that female-biased tumor growth involves multifaceted communications among tumor cells, hemocytes, and neuroendocrine insulin producing cells (IPCs). Notch-active tumor cells recruit hemocytes carrying the TNF-a homolog Eiger to the tumor microenvironment (TME), activating the JNK pathway in tumor cells, instigating the sexually-dimorphic upregulation of cytokine Unpaired 2 (Upd2). Upd2, in turn, exerts a distal influence by modulating the release of a *Drosophila* insulin-like peptide (Dilp2) from IPCs. Dilp2 then activates the insulin signaling in the tumor, thereby fostering sexual-dimorphic tumor growth. Together, these findings reveal a relay mechanism involving the TME and systemic signals that collectively control the sexual dimorphism of tumor growth.

232T **Meiotic crossover control at the *D. melanogaster* pericentromere is multifaceted** Nila M Pazhayam[1], Jeff Sekelsky[2] [1]Genetics and Molecular Biology, University of North Carolina at Chapel Hill, [2]UNC Chapel Hill

Crossing-over between homologous chromosomes is a critical part of meiosis that promotes proper chromosome segregation. The positioning of meiotic crossovers (COs) is intricately regulated via patterning events such as the centromere effect (CE), which ensures CO exclusion in pericentromeric regions. Although crucial to the meiotic cell, the mechanisms driving the CE are poorly understood in *D. melanogaster*.

The pericentromeric region of *D. mel* consists of two distinct types of heterochromatin - highly-repetitive alpha-heterochromatin and less-repetitive beta-heterochromatin. A previous study from our lab has shown that the CE is distinct in the two types of heterochromatin: alpha-heterochromatin displays a complete suppression of COs while beta-heterochromatin and proximal-euchromatin display a distance-dependent suppression, indicating that distinct processes may be responsible for the CE in each chromatin class. To further characterize CO control in these regions, we mapped centromere-proximal COs in WT flies as well as two classes of CE mutants – meiotic and structural - to proximal euchromatin, beta-het, or alpha-het. This allows us to ask whether different types of CE disruption affect different chromatin classes in the pericentromere, and whether multiple layers of CO control are required to effectively suppress COs in the region. The meiotic mutants in our study are mutants of *mei218* and *rec,* genes crucial for meiotic CO formation but with no significant roles outside of meiosis. The structural mutant in our study is a mutant of *Su(var)309*, the H3K9 methyltransferase necessary for pericentromeric heterochromatin formation. A synaptonemal complex (SC) mutant with CE defects was also included, as the SC is a structural component of meiosis, thereby straddling the line between both CE mutant classes in this study.

We observe significant redistribution of proximal COs from proximal-euchromatic regions into beta- as well as alpha-het in both meiotic mutants in our study, suggesting that meiotic pro-CO genes are necessary to prevent COs within pericentromeric heterochromatin. Surprisingly, our chromatin mutant – *Su(var)3-9*^{null} – did not display this redistribution from proximal-euchromatin into either class of heterochromatin, implying that meiotic machinery is more important in suppressing pericentromeric COs than the chromatin state of the pericentromere. This also suggests that meiotic COs in heterochromatin are prevented because they are meiotic COs and not because the region is heterochromatic. Additionally, our SC mutant displayed significant redistribution of COs from proximal-euchromatin into beta-heterochromatin, but not alpha-heterochromatin, suggesting that CO control within each pericentromeric chromatin class seems to be facilitated differently, with multiple facets acting in tandem to prevent COs within the entire region.

233T **Basal (non-induced) autophagy during meiotic prophase is required for accurate chromosome segregation in Drosophila oocytes** Diana C Hilpert, Muhammad Abdul Haseeb, Sharon E Bickel Biological Sciences, Dartmouth College

With increasing maternal age, chromosome segregation errors in human oocytes become more frequent, leading to a higher risk of aneuploid pregnancies and miscarriages in older women. Several lines of evidence indicate that sister-chromatid cohesion weakens as oocytes age and that premature loss of cohesion contributes to age-induced segregation errors. However, the underlying mechanism(s) that drive loss of cohesion in aging oocytes are not well-defined. Many aspects of cell function decline with age, including autophagy, a fundamental process that allows cells to degrade damaged organelles and protein aggregates and to survive periods of starvation. Here we use Drosophila oocytes as a model system to explore the possibility that non-induced (basal) autophagy is required for accurate meiotic chromosome segregation under normal feeding conditions. Using immunofluorescence to identify and quantify endogenous autophagosomes and autolysosomes in oocytes of three different wild-type strains, we confirmed that Drosophila oocytes perform basal autophagy under non-starvation conditions. In addition, using a Gal4/UAS approach to knock down individual autophagy proteins during meiotic prophase, we have found that autophagy during prophase I is required to promote accurate segregation in Drosophila oocytes. Moreover, the increased missegregation of recombinant homologs that we observe in Atg8a knockdown (KD) oocytes is consistent with premature loss of arm cohesion. Experiments utilizing Fluorescence In-Situ Hybridization (FISH) to directly assay the state of cohesion in Atg8a KD and control oocytes are currently underway. Our laboratory previously established a protocol that causes Drosophila oocytes to age and has shown that aging causes a significant increase in meiotic segregation errors. Therefore, this approach is able to recapitulate at least some aspects of human oocyte aging. We have used this aging method to compare basal autophagy in aged versus non-aged Drosophila oocytes. Our preliminary analysis indicates that that the percentage of oocyte volume occupied by autophagosomes and autolysosomes is significantly lower in Drosophila oocytes that have undergone aging. These data suggest that basal autophagic activity declines with age in Drosophila oocytes and may contribute to premature loss of cohesion and/or chromosome segregation errors in aging oocytes.

234T **Spontaneous meiotic nondisjunction of chromosome *2*: a genomic analysis** Carolyn Turcotte[1], Jeff Sekelsky[2] [1]Genetics and Molecular Biology, University of North Carolina at Chapel Hill, [2]Biology, University of North Carolina at Chapel Hill

During meiosis, crossovers between homologous chromosomes ensure proper chromosome segregation. To this end, the number and spatial arrangement of crossovers (termed “crossover patterning”) must be tightly regulated, and failures in crossover patterning can lead to aneuploidy and miscarriage. Despite its observation over 100 years ago, the mechanism behind crossover patterning, as well as the types of patterning defects that result in aneuploidy, remain poorly understood. Here, I interrogate crossover patterning in the context of meiotic nondisjunction to determine differences in crossover patterning between oocytes that faithfully segregate their chromosomes and oocytes that suffer chromosome missegregation (nondisjunction). I use a genetic trick to exclusively select *Drosophila melanogaster* offspring that are the direct product of oocytes that experienced chromosome *2* nondisjunction events, which are normally exceedingly rare (<0.1% of offspring). I then perform whole-genome sequencing to determine where crossovers occurred.

Consistent with prior interrogation of nondisjunction events on the *X* chromosome (Koehler et al. 1996), the majority of events were meiosis I nondisjunction. However, while most of the *X* chromosome nondisjunctional progeny did not receive a crossover on the *X* chromosome, most chromosome 2 nondisjunctional progeny did have at least one crossover on chromosome 2, and the observed crossovers did not have abnormal patterning compared to crossovers in cases of accurate chromosome segregation. These data suggest that the factors contributing to nondisjunction of different chromosomes may not be the same. Current directions include analyzing nondisjunction in the presence of balancer chromosomes, which have a multifactorial impact on nondisjunction in that 1) reduced recombination between the balancer and its homolog increases recombination on unbalanced chromosomes (the «interchromosomal effect»), and *2*) may increase mispairing and missegregation of chromosome *2*, as balancer chromosomes that do not pair with their homolog provide an alternate pairing partner when chromosome *2* does not receive a crossover.

235T **HP1 recruits the chromosomal passenger complex to the chromosome for acentrosomal spindle assembly in meiosis** Siwen Wu, Ryan D Doherty, Manisha Persaud, Keara Greer, Janet Jang, Kim S McKim Rutgers University

Chromosome segregation fidelity during female meiosis is critical for genome integrity, with aberrations causing infertility, miscarriages, and congenital anomalies. The chromosomal passenger complex (CPC)—composed of inner centromere protein (INCENP), Borealin, Survivin, and Aurora B kinase—regulates spindle assembly and ensures accurate chromosome segregation during meiotic cell division. In *Drosophila* oocytes, the CPC is required for microtubule recruitment to chromosomes, enabling acentrosomal spindle formation post-nuclear envelope breakdown during meiotic metaphase I. However, the mechanisms of CPC chromosomal recruitment and its interaction with spindle microtubules remain unclear. INCENP is a scaffolding component of CPC interacting with microtubules (MTs) and heterochromatin protein-1 (HP1). From our previous study, we hypothesized that HP1 recruits the CPC to chromosomes to initiate acentrosomal spindle assembly. While HP1 is present during prophase, the CPC was absent, indicating that CPC recruitment occurs after HP1. We then developed *HP1* RNAi reagents and generated *Incenp* mutants with deletions in HP1 binding sites to determine HP1's role in meiotic spindle organization and chromosomal biorientation. In *HP1* RNAi *Drosophila* oocytes, HP1 expression remained stable in early prophase but decreased in late prophase. By metaphase, HP1 levels further declined in *HP1* RNAi oocytes, and we observed increased chromosome segregation errors, suggesting HP1 has a crucial role in regulating chromosome biorientation. In addition, 30% of *HP1* RNAi oocytes failed to assemble spindles, indicating that HP1 has a role in spindle assembly. *Incenp* mutants lacking the HP1 binding domain showed increased chromosome segregation errors, but normal bipolar spindle formation, indicating that HP1-INCENP interaction is not responsible for spindle formation but does have a role in chromosome biorientation. Strikingly, we observed a complete failure of spindle assembly and CPC chromosomal recruitment in oocytes with the deletion of two domains, HP1 and SAH. These results suggest that multiple INCENP domains may be required to interact with HP1 and initiate spindle assembly in oocytes. We are currently investigating the mechanism for the movement of the CPC from the chromosomes to the microtubules.

236T **Fox transcription factors mediate proper positioning of cardiac cells by restricting the expression of ECM genes.** Rajnandani Katariya[1,2,3], Manoj Panta[1,2], Andrew J Kump[1,2,3], Neal Jeffries[4], Shaad M Ahmad[1,2,3] [1]Department of Biology, Indiana State University, [2]The Center for Genomic Advocacy, Indiana State University, [3]Rich and Robin Porter Cancer Research Center, Indiana State University, [4]National Heart, Lung and Blood Institute, NIH

The development of a complex organ requires the specification of appropriate numbers of its constituent cell types as well as their correct positioning within the organ. We previously showed that Fox transcription factors (TFs) Checkpoint suppressor 1-like (CHES-1-like) and Jumeau(Jumu) determine the correct number of different cardiac cell types by regulating cardiac progenitor cell divisions. Here we show that CHES-1-like and jumu are also required for the correct positioning of these cardiac cell types: null mutations in either gene result in the misalignment and incorrect location of cardial cells within individual hemisegments. Since defective divisions of cardiac progenitor cells in CHES-1-like and jumu loss-of-function mutants often lead to individual hemisegments having different numbers of cardial cells compared to their counterparts across the dorsal midline, we first examined whether the steric constraints caused by this asymmetry were a potential cause of incorrect positioning. Indeed, in Fox mutants, consistent with our hypothesis, contralateral hemisegments with unequal numbers of cardial cells showed significantly more positioning defects than contralateral hemisegments with the same number of cardial cells. However, our statistical analysis also showed that steric constraints could not explain all of the positioning defects: contralateral hemisegments with equal number of cardial cells in Fox mutants showed significantly more positioning defects than those with equal numbers from wild-type embryos. In order to discover the other cause underlying positioning defects, we compared genome-wide transcription expression profiles of purified mesodermal cells from wild-type embryos and Fox mutants to identify Fox-regulated targets. Among the 2,131 target genes we identified, genes encoding extracellular matrix (ECM) proteins were overrepresented among genes repressed by the Fox TFs. In particular, the ECM proteins Viking, Collagen type IV alpha 1, and Terribly reduced optic lobes were all overexpressed in Fox mutants. Our preliminary phenotypic analysis of these specific targets suggests that the Fox TFs bring about the correct positioning of cardiac cell types by restricting their expression: ectopic overexpression of each of these ECM genes in the mesoderm phenocopies the cardiac cell positioning defects observed in CHES-1-like and jumu loss-of-function mutants.

237T **Twin role of zinc finger transcription factor Castor: specification of cardiac cell subtype and regulation of cardiac progenitor cell division.** Rajnandani Katariya[1,2,3], M. Rezaul Hasan[1,2,3], Melissa Spognardi[2,4], Abbigayle J Gamble[1,2], Manoj Panta[1,2], Andrew J Kump[1,2,3], Shaad M Ahmad[1,2,3] [1]Department of Biology, Indiana State University, [2]The Center for Genomic Advocacy, Indiana State University, [3]Rich and Robin Porter Cancer Research Center, Indiana State University, [4]Saint Mary-of-the-Woods College

Mutations in the zinc-finger transcription factor-encoding gene CASZ1 lead to aberrant heart development in humans, Xenopus, and mice, indicating its conserved role in cardiogenesis. Our analysis of null mutations of castor (cas), the Drosophila ortholog of CASZ1, reveals that casplays two distinct roles in heart development. First, cas is required for mediating all three types of cardiac progenitor cell division: asymmetric, symmetric, and cell divisions at an earlier stage of development. Second, cas prevents subsets of cells in the most anterior region of the heart, the anterior aorta, from being specified as seven up-expressing cardial cells (Svp-CCs). In wild-type embryos, Svp-CCs are found exclusively in the posterior aorta and the even more posterior heart proper, regions of the heart determined by the expression of the Hox genes Ultrabithorax (Ubx) and abdominal A (abd-A). Intriguingly, both Ubx and abd-A repress cas, and ectopic expression of either of these two Hox genes in the anterior aorta leads to the ectopic specification of Svp-CCs there—a result which phenocopies cas loss-of-function mutants. Collectively, these data raise the possibility that that Ubx and abd-A specify Svp-CCs in the posterior aorta and the heart proper by repressing cas in those regions. In contrast, in the anterior aorta, in the absence of both Ubx and abd-A, cas levels may be sufficiently high to repress the Svp-CC fate. We are presently testing this hypothesis for cas-mediated Svp-CC specification and attempting to elucidate the pathways through which casregulates cardiac progenitor cell division.

238T **Mud at Meiosis: Don't worry about the Tip** Tara M Finegan, Dan T Bergstralh Biology, University of Missouri

The microtubule regulator Mud/NuMA/LIN-5 (flies/vertebrates/worms) is well-studied for its role in mitotic spindle orientation, a process that determines daughter cell placement and in some cases daughter cell fate. Mud is also observed in the fly oocyte, where it has been implicated in functions that include synapsis, nuclear migration, and spindle cohesion at meiosis II. How does this protein work? One way to approach this problem is by identifying conserved amino acid sequences in different orthologs. Sequence similarity between Mud orthologs is low, with the notable exception of a relatively short stretch called the NLM sequence (so named because it is found in NuMA, Mud, and LIN-5) that is thought to include both a microtubule-binding domain and the binding site for an important partner protein called Pins/LGN/GPR1-2 (flies/vertebrates/worms). Remarkably, flies encode isoforms that do not include the NLM. This raises two questions: what does the NLM sequence do, and what functions of Mud don't require it? Our work suggests that the isoforms lacking the NLM participate in meiosis. We show that these isoforms are expressed in the testis and ovary but not in the somatic tissues we examined. Additionally, whereas Mud has established roles in the oocyte, we show here that it is also at the nuclear envelope of developing sperm. We are now investigating the question of how isoforms lacking the NLM work

239T **Elucidating the role of Polo kinase activity and regulation in the meiotic drive of B chromosomes in *D. melanogaster*** Kaylah B Samuelson[1,2], Mengjia M Lin[1,2], Ryan Gado[1,3], Allison Gardner[1], Abigail Goldhamer[1], Annette St. Jacques[1], Stacey L Hanlon[1,2] [1]Molecular and Cell Biology, University of Connecticut, [2]Institute for Systems Genomics, University of Connecticut, [3]New York University

Recently discovered B chromosomes in *D. melanogaster* are transmitted through female meiosis at a higher-than-expected frequency, a phenomenon known as meiotic drive. Interestingly, this drive is dependent on a genetic reduction in *matrimony* (*mtrm*), a key regulator of female meiosis. Mtrm normally works with Polo kinase (Polo) in a 1:1 genetic ratio to regulate chromosome segregation, leading us to speculate that the maintenance of this ratio is important to suppress the drive of the B chromosomes. To test this, we genetically altered the ratio of *mtrm* and *polo* and found that B chromosome transmission decreases as the genetic levels of Mtrm increase relative to Polo, suggesting that a high Polo:Mtrm ratio is necessary to promote drive of the B chromosomes. Since the direct interaction of Polo and Mtrm has been previously shown to be essential for proper meiotic chromosome segregation, we also tested if this interaction is necessary to suppress the drive of the B chromosomes. We found that B chromosome transmission is still high in a *mtrm/+* heterozygote that expresses a mutant allele of Mtrm that cannot bind Polo ($mtrm^{T40A}$), indicating that the direct interaction of Mtrm and Polo is necessary to suppress drive. Based on our preliminary observations of chromosome dynamics during the female meiotic divisions, we hypothesize that the increase in Polo activity results in the premature separation of sister chromatid cohesion, leading to lagging B chromosomes during anaphase I and II. To explore if regulation of Polo through alternative pathways can also influence the drive of the B chromosomes, we are currently assessing B chromosome transmission in the presence of a hypermorphic allele of Greatwall (Gwl^{Scant}), a meiotic and mitotic kinase that antagonizes Polo activity independent of Mtrm. Overall, our investigation has revealed how the activity and regulation of Polo kinase during female meiosis promotes Mendelian chromosome segregation and prohibits the drive of B chromosomes in *D. melanogaster*.

240T **Multiple maternal factors induce the mitotic failure of the *359-bp* satellite in *Drosophila* hybrids** Tianzhu Xiong[1], William J Furnas[2], Dean Castillo[3], Daniel A Barbash[1] [1]Molecular Biology and Genetics, Cornell University, [2]Cornell University, [3]Miami University

Satellite DNA can be fragile in mitosis. One example is the *359-bp* satellite, which forms a large pericentric heterochromatic block on the X chromosome of *D. melanogaster*. Interspecific female F_1 hybrids from *D. simulans* females crossed to *D. melanogaster* males are embryonic lethal, although the penetrance of lethality varies widely among *D. simulans* strains. This lethality results from the *D. melanogaster* X chromosome sister chromatids entangling throughout the *359-bp* satellite and failing to segregate at anaphase, indicating a lethal interaction between the *359-bp* satellite and maternal factors in *D. simulans*. However, these maternal factors have remained obscure because hybridization rarely occurs in this cross, rendering QTL mapping prohibitive. Here, we used a highly recombined pool of *D. simulans* formed by intercrossing two strains with opposite levels of hybrid lethality for over 50 generations. We partially overcame the mating barrier with environmental stimuli and sequenced 183 recombinants associated with either no lethality or full lethality in their hybrid female offspring. QTL mapping with these recombinants revealed multiple loci on multiple chromosomes acting together to cause lethality. Our mapping result is congruent with the observed continuous natural variation in *D. simulans* for F_1 hybrid female lethality.

241F **The Ptch/SPOUT1 methyltransferase deposits an m^3U modification on 28S rRNA for normal ribosomal function in flies and humans** jie chen[1], yaofu bai[1,1], yuantai huang[2], min cui[1], yiqing wang[1], zhengqi gu[1], xiaolong wu[1], yubin li[3], Yikang Rong[1] [1]university of south china, [2]sun yet-sen university, [3]jinzhou medical university

The ribosomal RNA (rRNA) is one of the most heavily modified RNA species in nature. Although we have advanced knowledge of the sites, functions and the enzymology of many of the rRNA modifications from all kingdoms of life, we lack basic understanding on many of those that are not universally present. A single N^3modified uridine base (m^3U) was identified to be present on the 28S rRNA from humans and frogs, but absent in bacteria or yeast. Here we show that the equivalent m^3U is present in Drosophila, and that the Ptch/CG12128 enzyme and its human homolog SPOUT1 are both necessary and sufficient for carrying out the modification. The Ptch-modified U is at a functional center of the large ribosomal subunit, and consistently *ptch*-mutant cells suffer loss of ribosomal functions. SPOUT1, suggested to be the most druggable RNA methyltransferases in human, represents a unique target where ribosomal functions could be specifically compromised in cancer cells.

242F **Synaptic Vesicle Glycoprotein 2 function during multipolar division in *Drosophila melanogaster*** Jane E Blackmer[1], Don Fox[2] [1]Molecular Cancer Biology, Duke University School of Medicine, [2]Duke University

Centrosome number Amplification (CA), or >2 centrosomes per cell, can lead to multipolar spindle formation, multipolar division, and aneuploidy of the daughter cells. CA therefore poses a distinct problem for dividing cells, as aneuploidy can drive cell death. Clustering of extra centrosomes allows cells to undergo pseudo-bipolar division into two daughter cells, therefore avoiding aneuploidy and cell death. However, certain cell types, including the rectal papillar cells of *Drosophila*, often fail to cluster their extra centrosomes, but can tolerate the resultant multipolar division and aneuploidy. Additionally, CA is a common cancer phenomenon, and identification of factors necessary for centrosome clustering-independent survival of cells would provide insight not only into basic cellular biology, but also into mechanisms of cancer cell survival.

To identify factors of clustering-independent cell survival, we performed a forward genetics screen in the *Drosophila* papillar cells. We mutagenized 6,617 fly lines with EMS and selected for homozygous lethal mutations on the X chromosome involved in clustering-independent survival. Through a combination of duplication and transgene rescue, we identified the conserved gene Synaptic Vesicle Glycoprotein 2 (SV2) as necessary for clustering-independent survival of cells with CA. Furthermore, knock-down of SV2 during mitosis recapitulates the mutant phenotype of decreased papillar cell numbers, and concurrent knockdown of SV2 with CA synergizes to create a more severe phenotype than either alone. To our knowledge, SV2 has never been studied in cell division. Through GFP-tagging of SV2, we have shown the protein to localize to the cell membrane in multiple cell types, including papillar cells. Live imaging of papillar cells during mitosis with SV2 RNAi and CA shows severe mitotic defects during multipolar divisions including chromosome misalignment and lagging chromosomes leading to micronuclei, centrosome mispositioning, membrane instability and cytokinesis failure, and uneven multipolar anaphase. We predict that SV2 interacts with the spindle to ensure mitotic integrity, including the correct positioning of centrosomes via astral microtubules, the correct alignment of chromosomes via kinetochore microtubules, and the establishment of the cleavage furrow with interpolar kinetochores. Further study of SV2's role in multipolar division could provide further insights into cell biology and cancer cell vulnerabilities.

243F **Hippo-activated cells induce non-cell autonomous tumorigenesis in *Drosophila*** Daichi Honda[1], Misako Okumura[2], Tomoki Umehara[3], Chisako Sakuma[4], Toshinori Ando[5], Masayuki Miura[3], Takahiro Chihara[2] [1]Program of Biomedical Science, Graduate School of Integrated Sciences for Life, Hiroshima University, Japan, [2]Graduate School of Integrated Sciences for Life, Hiroshima University, Japan, [3]Graduate School of Pharmaceutical Sciences, The University of Tokyo, Japan, [4]RIKEN Center for Biosystems Dynamics Research, Japan, [5]Center of Oral Clinical Examination, Hiroshima University Hospital, Japan

The Hippo pathway is known as the tumor suppressor pathway, and most of the related studies indicate that inactivation of the Hippo pathway leads to tumorigenesis. However, the genetic analysis has revealed that the Hippo pathway is not frequently mutated and even activated in some cancers, suggesting that the activated Hippo pathway may contribute to tumorigenesis. Therefore, we decided to investigate the paradox of the Hippo pathway: the possibility that activation of the Hippo pathway induces tumorigenesis.

First, we genetically induced Hippo-activated cells in the wing disc by altering a gene expression of the Hippo-pathway components (*strip*, *warts*, and *yorkie*). Then, we examined the effects on cancer markers, the Ribosomal protein S6 phosphorylation (a marker of tissue growth), EDU labeling (a marker of proliferation), and MMP1 expression (a marker of invasion). Hippo-activated cells upregulated these cancer markers in their surrounding cells, thereby transforming the surrounding cells into "cancer-like cells". This suggests that Hippo-activated cells become "oncogenic niche cells". Second, we examined the downstream target of the Hippo pathway required for non-cell-autonomous tumorigenesis. We found that the expression level of *hid* (an apoptotic inducer), *atg8a* (an autophagy-related gene), and *cyclin E* (a proliferative activator) in the Hippo-activated cells are crucial for non-cell autonomous tumorigenesis. Then, we found that Hippo-activated cells would upregulate and secrete growth factors like Wg and Spitz for non-cell autonomous tumorigenesis through Hid/Dronc/Rho1 signaling. However, activation of Hid/Dronc/Rho1 signaling alone could not phenocopy non-cell-autonomous tumorigenesis. Thus, we predicted that different secretory molecules in addition to Wg and Spitz would be used and secreted by Hippo-activated cells. Finally, we conducted genomic deficiency screening to identify regulators of non-cell-autonomous tumorigenesis. We discovered the evolutionarily conserved amino acid transporters that we named *sat1/2*. *sat1/2* knockdown in Hippo-activated cells significantly suppressed non-cell-autonomous tumorigenesis. We hypothesize that Hippo-activated cells use amino acids in addition to Wg and Spitz for non-cell-autonomous tumorigenesis. Our study can give a novel insight into the Hippo pathway in tumorigenesis.

244F **Investigating the potential role of Mapmodulin as an H2A.Z chaperone in regulating early embryonic development** Noah Reger, Patrick Murphy, Michael Welte University of Rochester

Early embryos have an abundance of cytoplasmic H2A.Z that, as the embryo approaches the midblastula transition (MBT) at nuclear cycle 13 (NC13), is transferred to the nuclei. We have found embryos with an overabundance of nuclear H2A.Z have an accelerated NC13, increased DNA damage, and changes in the transcriptome, suggesting that exact nuclear H2A.Z levels are critical for development. These levels are, in part, controlled by Jabba which sequesters H2A.Z on cytoplasmic lipid droplets. However, it remains unclear whether nuclear H2A.Z levels are maintained by additional mechanisms. One promising candidate is Mapmodulin, the sole ortholog of vertebrate Anp32e. Anp32e is a known H2A.Z interactor, and published proteomic data as well as our own structural modeling suggests the same is true for Mapmodulin. Our goal is to determine if Mapmodulin regulates nuclear H2A.Z in fly embryos. Using several protein trap lines that fluorescently tag Mapmodulin, we found that Mapmodulin localizes to both nuclei and the cytoplasm, but nuclear enrichment is cell-cycle dependent. During S phase, Mapmodulin is highly enriched in the nucleus relative to the cytoplasm. In mitotic nuclei, Mapmodulin nuclear intensity decreases and the remaining signal is nucleoplasmic but not obviously associated with chromatin. We also observed that embryos with Mapmodulin tagged near the C-terminus have a higher nuclear-to-cytoplasmic ratio compared with those tagged near the N-terminus. This suggests that Mapmodulin localization is sensitive to location of tagging. Interestingly, there is a predicted NLS and phospho-site in the C-terminus. We hypothesize that tagging of Mapmodulin near the C-terminus disrupts phosphorylation leading to overaccumulation of Mapmodulin in the nucleus. These data suggest Mapmodulin may serve a cell-cycle dependent function in the embryo. In vertebrates, one proposed model argues that Anp32e acts as a histone evictor, removing H2A.Z from the chromatin. Another model for Anp32e proposes it sequesters H2A.Z in the nucleoplasm and cytoplasm, preventing incorporation in the chromatin. We hypothesize that Mapmodulin functions in the early embryo to sequester H2A.Z in the nucleus to prevent incorporation of H2A.Z into the chromatin. To test this, we are generating Mapmodulin mutants and will test whether loss of Mapmodulin yields similar results as loss of Jabba.

245F **Mitotic polarity oscillation promotes epithelial tumor progression.** Gayaanan Jeyanaathan[1], Ming Meggie Cao[1], Milena Pellikka[1], Parama Talukder[1], Sarah JL Robinson[2], Vanessa B Ghorayeb[2], Ulrich Tepass[1] [1]University of Toronto, [2]Cell and Systems Biology, University of Toronto

Mitosis of epithelial cells requires a transient loss of epithelial polarity. However, the nature of this mitotic polarity oscillation and its functional consequences for epithelial development are not fully understood. Here we show that the Crumbs (Crb) complex, a key regulator of epithelial polarity, is lost from the membrane during mitosis, and the Crb mutant phenotype is ameliorated when cell division is inhibited. Remarkably, an essential requirement of Crb for epithelial polarity is fully suspended when cell division is blocked in conjunction with inhibition of either cell ingression or cell intercalation. We conclude that the amount of morphogenetic stress induced by mitosis, ingression, and intercalation determines the requirement for Crb. Increased cell division and loss of cell polarity are two main drivers of epithelial cancer. Maintaining epithelial polarity is important for limiting proliferation. Whether the loss of polarity during mitosis impacts tissue growth is less clear. We show that increasing cell division in a morphogenetically quiet epithelium not only increases tissue size but also causes hyperplastic to neoplastic transition. Conversely, reducing cell division restores epithelial polarity in neoplastic tissue of tumour mutants. Taken together, our study revealed that a major function of polarity factors in epithelial maintenance is to counteract morphogenetic stress. Moreover, we propose a feedforward mechanism that links cell division and the loss of polarity as a key driver of epithelial cancer.

246F **Intercellular organelle transport in the larval adipocytes is mediated by ring canals** Shyama Nandakumar, Deepika Vasudevan Cell Biology, University of Pittsburgh

Drosophila melanogaster development from embryo to late larvae involves exponential growth over a short period. Most of this is supported by the rapid growth of the fat body, which is responsible for energy storage, nutrient dissemination, hormone secretion as well as innate immune responses. How adipocytes in the fat body communicate with each other to execute such complex systemic events is poorly understood. We discovered that adipocytes are each paired to one neighboring cell through intercellular bridges or ring canals. Ring canals, which form as a result of an incomplete cytokinesis have been extensively characterized in the adult germline, and adult somatic tissues of the reproductive system but their function in larval adipocytes is unknown. Using super-resolution microscopy, we determined the dimensions of ring canals in the fat body, and show that they do not grow in size throughout larval development, even as the adipocytes grow exponentially by endoreplication . Additionally, we used fluorescence recovery after photobleaching to show that fat body ring canals permit transport of organellar cargo including endoplasmic reticulum, Golgi vesicles and cytoplasmic calcium. However, we do not observe transport of mitochondria, suggesting that these ring canals are cargo selective.

We previously demonstrated that the larval fat body tolerates high levels of intrinsic endoplasmic reticulum (ER) stress due to its high metabolic load. Using an ER stress reporter (4E-BP^{intron}-DsRed), we find that adipocytes displaying higher levels of stress are more likely to engage in ring canal-mediated transport of cytoplasmic material. Depletion of the ER stress response transcription factor, ATF4, disrupted cargo transport across paired adipocytes. we hypothesize that adipocyte ring canals play an important role in maintaining fat body homeostasis by buffering acute stress response. Our ongoing experiments are testing this in addition to determining the structural components of the ring canals.

The larval fat body undergoes apoptosis and transformation during metamorphosis; recent work from other groups has shown that pupal progenitors give rise to the adult fat body which contains smaller cells which fuse to give rise to multinucleate adipocytes. We find that adult adipocytes do not possess ring canals. However, both the larval and the adult fat body contain syncytial cells - the larval fat body comprising several binucleate syncytia connected via ring canals, and the adult fat body containing binucleate and tetranucleate syncytial cells as a result of cell fusion. This suggests that context dependent variation in multinucleation & syncytialization is an important facet of *Drosophila* adipocytes. I will present our ongoing work in understanding the role of multinucleation in adipocyte metabolism & stress tolerance.

247F **The Characterization of *Drosophila* FANCD2 in Repair of DNA Double-strand breaks** Jeannine R. LaRocque[1], Shagun Gandhi[1], Elizabeth Vesialou[2] [1]Human Science, Georgetown University, [2]Georgetown University

Interstrand DNA crosslinks (ICLs) are toxic forms of DNA damage that stall replication and transcription by covalently binding both strands of the DNA double-helix. Fanconi Anemia (FA) proteins repair ICLs during S phase via a double-strand break (DSB) intermediate to maintain genome stability. Although there have been advances in understanding the role of FANCD2 in the repair of ICLs, there is still limited information on the role FANCD2 plays in the repair of DSBs unrelated to ICLs in multicellular organisms. Thus, this research analyzes the role of *Drosophila* FANCD2 in DSB repair. We find that *FANCD2* null (*FANCD2*$^{\Delta}$) and ubiquitin-dead (*FANCD2*^{K595R}) alleles are sensitive to ionizing radiation in a dose-dependent manner. We also investigated the role of FANCD2 in repair pathway choice (non-homologous end joining vs. homologous recombination) in the premeiotic germline and the somatic tissue of *Drosophila* using the DR-*white* assay and the Tracking of Indels by DEcomposition (TIDE) algorithm, respectively. The premeiotic germline and somatic tissue data suggests that there is a statistically significant decrease in repair by HR in the *FANCD2*$^{\Delta}$ null and *FANCD2*^{K595R} ubiquitin-dead mutants compared to the heterozygote controls. We conclude that DSBs unrelated to ICLs may require FANCD2 for their repair through the HR pathway.

248F **A potential role of NudC in ribosome biogenesis homeostasis in *Drosophila* polyploid cells** Duoduo Shi[1], Yuko Shimada-Niwa[2], Naoki Okamoto[2], Yuya Ohhara[3], Akira Nakamura[4], Wei Sun[5], Ryusuke Niwa[2] [1]Graduate School of Science and Technology, University of Tsukuba, Japan, [2]Life Science Center for Survival Dynamics, Tsukuba Advanced Research Alliance (TARA), University of Tsukuba, Japan, [3]Laboratory of Human Genetics, School of Food and Nutritional Sciences, University of Shizuoka, Japan, [4]Institute of Molecular Embryology and Genetics, Kumamoto University, Japan, [5]School of Life Sciences, Chongqing University, China

Ribosomes, the cellular machinery responsible for protein synthesis, are fundamental to life and cell growth. Disruptions in ribosome biogenesis can lead to severe disorders known as ribosomopathies, underscoring the importance of the regulatory mechanisms in this process. This study shows that the gene *NudC* (*nuclear distribution C, dynein complex regulator*) is a potential key player in ribosome biogenesis in polyploid cells, particularly in *Drosophila* larval salivary gland cells. While NudC is known to be involved in nuclear migration and cytokinesis in mitotic cells, its function in post-mitotic cells has remained elusive. Here, we show that NudC plays a crucial role in maintaining ribosome biogenesis in post-mitotic polyploid cells. Knockdown of *NudC* via RNAi in salivary gland cells resulted in striking cellular abnormalities, including loss of the endoplasmic reticulum, abnormal ribosome distribution, and a significant reduction in 28S ribosomal RNA (rRNA). These changes were accompanied by a cascade of downstream effects typically associated with ribosome loss, such as mRNA degradation, JNK-dependent autophagy, accumulation of virus-like particles, and abnormal chromosome structure. Intriguingly, our data suggest that NudC's role in ribosome biogenesis may be independent of its known function in dynein regulation, suggesting potential 'moonlighting' functions for this protein. We also observed a homeostatic mechanism of ribosome biogenesis-related gene expression triggered by NudC depletion. *NudC* RNAi cells showed substantial up-regulation of ribosome biogenesis factors and increased expression of ribosomal proteins, mirroring the response observed in cells depleted of known ribosome biogenesis factors. This similarity suggests a potential collaborative role between NudC and ribosome-related proteins in maintaining ribosome biogenesis homeostasis. In conclusion, our study of *Drosophila* larval salivary gland cells reveals a potential 'moonlighting' function for NudC in maintaining ribosome biogenesis homeostasis. Ongoing research aims to unravel the precise mechanisms by which NudC regulates rRNA levels and maintains ribosome homeostasis in these specific cell types, potentially providing insights into similar processes in other organisms.

249F **A reverse genetic screen for ploidy-specific gene function** Joshua Silva[1], Yi-Ting Huang[2], Brian Calvi[2], Don Fox[1] [1]Pharmacology and Cancer Biology, Duke University, [2]Biology, Indiana University

From cancers to hepatocytes, to even the crops we consume, polyploidy (containing more than two genome sets in a cell) can be found in the cellular, tissue, and even organismal scales. Despite this cellular state's prevalence across numerous biological circumstances, a foundational understanding of the nucleotypic effects (effects due to DNA content) of whole genome duplication (WGD) as well as the genetic basis of these effects is lacking. We previously developed a *Drosophila* model that enables us to induce the formation of tetraploid cells by promoting one round of endocycles (duplication of genome without subsequent mitosis) in any cycling larval cells through manipulation of the anaphase promoting complex. This inducible system allows us to study the nucleotypic effects of tetraploid and diploid cells in the same tissue as well as create mixed ploidy organs. Previously, we observed no overt developmental defects in animals with induced tetraploidy, yet it was unclear whether altering ploidy impacted the requirement for specific genes. Using this system, we have performed a large-scale RNAi screen of the Bloomington TRiP collection for ~1600 genes. We screened for ploidy dependent regulators using the eye as a phenotypic readout. We found far more tetraploid-specific phenotypes than diploid-specific phenotypes, indicating that development of a tetraploid eye requires altered gene function. Among our tetraploid-specific hits, the most severe hits represented 7% of all genes screened. Our screen elucidated many previously unknown pathways that could be implicated in ploidy regulation and sets up many future avenues for investigation. In particular, we identified specific cell growth pathways having increased importance in tetraploid cells, as well as evidence for large scale metabolic rewiring occurring in tetraploid cells. Additionally, we are using this system to characterize differences between eye-antennae imaginal discs with only diploid cells and those with mixed diploid and tetraploid cell populations. We have found that in the eye imaginal disc there is bias towards polyploid cells (~75%) when compared to diploid cells just 48 hours after a short, singular induction of endocycles indicating a possible cell competition mechanism between diploid and tetraploid cells. Though this phenomenon of greater polyploidy fitness over diploid cells has been observed in cancer models, competition between ploidies during tissue growth has not been extensively studied. This work will allow us to ultimately identify unique properties of polyploid cells that have previously been unreported.

250F **B chromosomes disrupt proper chromosome segregation during female meiosis** Suparna Dutta[1], Shell Chen[2], Stacey L Hanlon[1] [1]Molecular and Cell Biology, University of Connecticut, [2]Molecular and Cell Biology, Brandeis University

The recently discovered B chromosomes in *Drosophila melanogaster* are nonessential, do not carry any known genic regions, and appear to be composed entirely of heterochromatin. Based on shared sequence elements, these B chromosomes are likely derived from chromosome 4. Interestingly, the B chromosomes can induce high levels of chromosome 4 missegregation during female meiosis, prompting us to investigate the interaction between chromosome 4 and the B chromosomes. We first examined B chromosome transmission during female meiosis when zero, one, or two copies of chromosome 4 are passed on to progeny. We observed an inverse relationship between B chromosome and chromosome 4 segregation: when the female passes on two copies of chromosome 4, fewer B chromosomes are transmitted, and when zero copies of chromosome 4 are passed on, more B chromosomes are transmitted. To further investigate the effect of B chromosomes on chromosome 4 segregation during female meiosis, we measured chromosome 4 nondisjunction as a function of B chromosome copy number. Our preliminary data with a small B chromosome copy number range indicate no significant correlation between B chromosome copy number and chromosome 4 nondisjunction. We are currently expanding this B chromosome copy number range to determine if there is still a lack of correlation between B chromosome copy number and chromosome 4 nondisjunction, or if there is a threshold for how many B chromosomes can be present during meiosis before they affect the segregation of other chromosomes. Additionally, we plan to explore the interaction between B chromosomes and chromosome 4 cytologically by examining heterochromatic threads. These threads form between homologous chromosomes during meiosis, and if they are improperly forming between the B chromosomes and chromosome 4, it may be affecting the proper distribution of chromosome 4 on the metaphase I spindle. Overall, our research aims to enhance our understanding of how supernumerary, nonessential chromosomes influence the segregation of essential chromosomes during female meiosis.

251F **Uncovering how increased dietary protein regulates the IGF-1 homolog Dilp6** Kelly E Dunham, Miyuki Suzawa, Michelle L Bland Pharmacology, University of Virginia

Nutrients are needed for animals to grow, and hormones like insulin and insulin-like growth factors are necessary to communicate their availability. *Drosophila melanogaster* possess seven *Drosophila* insulin-like peptides (Dilps), but the fly genome only encodes one insulin receptor (InR). Currently, it is unclear how or whether this InR discriminates among Dilps, how distinct Dilps respond to different dietary nutrients, and how Dilps mechanistically impact growth and metabolism. Using a nutritional geometry approach in larvae, we found that Dilp2 and Dilp6 respond oppositely to increased dietary sugar and protein. Interestingly, high protein decreased Dilp6 secretion but was necessary for whole animal growth. Food intake did not differ appreciably between diets with varying amounts of sucrose and yeast extract, although ingested energy differed greatly among diets. Using our diets, we are investigating how canonical pathways regulated by amino acid availability – the Gcn2-eIF2α and GATOR-mTORC1 pathways – may regulate Dilp6 gene expression and secretion. As expected, mTORC1 signaling increased with increased dietary protein, but we also found elevated sestrin gene expression, which may be responding to nutrient overload. Ultimately, our initial validation experiments show that RNAi specific knockdowns for these pathways will be successful to pinpoint how dietary protein impacts hormone signaling from juvenile to adult life stages.

252F **Identification of *sisters separate (ssep),* a gene required for meiotic centromere cohesion in *Drosophila melanogaster*** Sanay T Hewitt[1], Sarah Pellizzari[1], Sean Thornton[1], Hallett S Ward[1], Aaron Keat[1], John Tomkiel Dean[2] [1]Biology, Univ North Carolina Greensboro, [2]Univ North Carolina Greensboro

Cohesins regulate attachment between sister chromatids in both mitosis and meiosis. Two complexes of cohesins that differentially affect arm cohesion and centromere cohesion have been proposed. Sunn, Solo, and Ord are thought to regulate cohesion at centromeres in meiosis (Gyuricza 2016). Here we describe *sisters separate* (*ssep*), a new gene required for meiotic sister chromatid cohesion. Mutations in *ssep* cause early separation of sister chromatids in meiosis I leading to nondisjunction of both sex chromosomes and autosomes. Effects of mutations in *ssep* and *sunn* are not additive, suggesting they act in the same pathway. Similar to *sunn* and *solo* mutants, sister centromeres separate precociously in *ssep* mutants during stage 4 of spermatocytes (mid prophase I). A *bam-GAL4* driven *mcherry*- tagged *ssep* transgene rescues the nondisjunction and colocalizes with CID at centromeres until anaphase II. Centromere localization is not seen when Ssep is expressed in spermatocytes after stage 3, suggesting that loading is required earlier in meiosis and that there is no turnover. Mutation of a putative conserved Separase cleavage site abolishes CEN localization. These observations suggest that Ssep is a part of the sunn, solo, ord cohesin complex.

253S **Developing a Drosophila model for Neprilysin's role in cisplatin resistance** Jaxon C Salazar, Jennifer Curtiss Biology, New Mexico State University

Cisplatin is a chemotherapeutic drug that creates DNA crosslinks. These crosslinks can be repaired by the **Fanconi anemia pathway**, such that an upregulation of the Fanconi anemia pathway can result in cisplatin resistance. **Neprilysin (Nep)**, part of the M13 family of metallopeptidases has been linked to increased resistance to cisplatin in ovarian cancer stem cells. We created a Nep1 mutant model in *Drosophila melanogaster* using CRISPR/Cas9. RNA sequencing of these mutants, followed by functional enrichment analysis, showed significant upregulation of genes in the Fanconi anemia pathway (7.8-fold enrichment, P=0.00019), indicating a possible regulatory connection. We hypothesize that Neprilysin upregulates the Fanconi anemia pathway, generating cisplatin resistance. To test this hypothesis we are administering cisplatin to the Nep1 mutants and evaluating DNA damage and repair processes, changes in gene expression, and phenotypic outcomes.

254S **Polyploid cell migration depends on JNK activation** Youfang Zhou, Xianfeng Wang, Wu-Min Deng Tulane University

Polyploidy plays an essential role in both normal developmental and abnormal conditions in *Drosophila* and mammals. Given its importance in tissue function, we aimed to adjust polyploidy levels in normal tissues to better understand these functional distinctions. Border cells, a specialized group of migratory cells within the ovary, represent a classic example of polyploid cells in normal development. We found that reducing ploidy in border cells by expressing RNAi of *fzr* (fizzy-related, a positive regulator of the Anaphase-Promoting Complex/Cyclosome (APC/C)) led to migration defects. In contrast, increasing ploidy by expressing *fzr* and the CycA inhibitor *roughex* (rux) in the diploid wing imaginal disc resulted in polyploid cells. Moreover, these induced polyploid cells exhibited membrane extensions, JNK activation, and gained migratory ability. Further, inhibiting JNK activation by expressing a dominant-negative form of JNK (*bsk*-DN) significantly suppressed polyploid cell migration, demonstrating that polyploid cell migration relies on JNK. Interestingly, *bsk*-DN expression also led to polyploid cell death, suggesting that the JNK pathway supports polyploid cell survival. Our further investigation indicated that JNK activation is associated with reactive oxygen species (ROS) production. In summary, these findings reveal the role of polyploidy and JNK activity in facilitating cell migration under both natural and induced conditions.

255S **Transcriptional regulation in response to cell size during zygotic genome activation** Grace I Carey[1], Shufan Lin[2], Bomyi Lim[2], Amanda A Amodeo[1] [1]Biological Sciences, Dartmouth College, [2]Chemical and Biomolecular Engineering, University of Pennsylvania

Cell size changes drastically during development. In early *Drosophila* embryogenesis, several rounds of nuclear divisions occur without changing the embryo's size, increasing the nuclear-to-cytoplasmic ratio (N/C ratio). The changing N/C ratio determines the timing of key developmental processes, including zygotic genome activation (ZGA) and the slowing of the cell cycle during the mid-blastula transition. The activation of many zygotic genes relies largely on the slowing of the cell cycle, which lengthens the window in which transcription can occur. However, previous work uncovered a handful of genes that respond directly to the N/C ratio, including the mitotic inhibitor *fruhstart*. Other cell cycle regulators are known to respond to cell size in yeast, suggesting a potential feedback mechanism between the N/C ratio and cell cycle control. The molecular mechanisms that govern a gene's sensitivity to the N/C ratio remain unknown. To identify a minimal sequence that confers this behavior, we have used transgenic reporters to identify a 300bp portion of *fruhstart*'s cis-regulatory region that drives N/C ratio-sensitive expression. To understand the cis-regulatory components that give rise to N/C ratio sensitivity, we re-analyzed published RNA-seq data from cell cycle-arrested embryos to identify candidate genes that are activated at a specific N/C ratio. Our list of candidates includes genes regulating cell cycle, DNA replication, and chromatin remodeling. We hypothesize that a common regulatory mechanism allows such genes to sense the changing N/C ratio. To uncover this mechanism, we have performed DNA motif analysis on the regulatory regions of N/C ratio-sensitive genes to identify factors whose binding is enriched near these genes and could modulate their transcription. Preliminary results show an enrichment of binding motifs for the insulator-associated proteins BEAF-32 and Dref near N/C ratio-sensitive genes, suggesting that chromatin architecture may play a role in sensing the N/C ratio. Published ChIP-seq data also shows an enrichment of origins of replication near N/C ratio-sensitive genes, indicating that DNA replication might also contribute to N/C ratio sensitivity. Further investigation is necessary to determine if and how these pathways may contribute to the cell size-sensitive regulation of transcription.

256S **The Fanconi Anemia pathway regulates Nickase-induced HTR in *Drosophila*** Ian Rousseau, Liana Moricz, Ketta Sneider, Margot Mel de Fontenay, Annabel Guichard, Ethan Bier Biological Sciences, University of California San Diego

CRISPR-based applications for gene editing involve creation of an initial Double Strand Break (DSB) by Cas9 or a Single Strand Break (SSB or nick) produced by Cas9 variants D10A or H840A at a specific site defined by the sequence of a gRNA. Repair of such DNA lesions is accomplished through mutagenic NHEJ (Non-Homologous End Joining), which ligates the DNA strands back together, or by HDR (Homology-Directed repair), which employs a homologous sequence as a template and copies those sequences into the break in an error-free fashion. We have designed a system in which a targeted cut or nick at the *white* locus is repaired using the genetic information from the homologous chromosome to produce red clones in otherwise white eyes in *Drosophila*. This specific type of somatic repair is termed HTR, for Homologous chromosome-Templated Repair. We conducted an RNAi screen to identify DNA repair components involved specifically in HTR in which we selectively blocked expression of candidate DNA repair genes in the dorsal component of the eye, using the MirrGAL4 driver. Thus, the ventral portion of the eye provides an internal control to compensate for potential variations in genetic backgrounds. Our screen revealed 8 genes that either suppress or enhance HTR when blocked by RNAi. Interestingly, genes implicated in Nickase-induced repair are partially distinct from those involved in Cas9-induced repair. Among these are 3 genes from the Fanconi Anemia pathway that specifically affect Nickase based repair. This analysis sheds light on mechanistic aspects of HTR processes and could guide the design of future safe gene editing strategies to correct disease-causing alleles in human patients.

*Ian Rousseau and Liana Moricz are co-first authors

Roy et al, “Cas9/Nickase-induced allelic conversion by homologous chromosome-templated repair in *Drosophila* somatic cells”. *Sci. Adv.***8**,eabo0721(2022).

257S **Investigating the roles of Kinases and Phosphatases in Tumor Cell Dissemination** Ginger Chiu, Alan Vu, Trish Nguyen, Vincente Abatay, Nickolas Dominguez, Jiae Lee Biological Sciences, California State University of Long Beach

Tumor cell dissemination is a crucial step in initiating cancer metastasis, responsible for approximately 90% of cancer-related deaths. This process is regulated by various signaling pathways, many of which are controlled by protein phosphorylation and dephosphorylation that regulate protein activity, mediated by kinases and phosphatases, respectively. Despite their importance, the signaling pathways that cause tumor cell dissemination remain largely unknown. In this study, we utilize *Drosophila melanogaster* as an in vivo intestinal tumor model, driven by the expression of the oncogene Ras^{V12} in intestinal stem cells, to investigate kinase and phosphatase roles in tumor dissemination within a native tissue context. This model allows precise temporal and spatial regulation of gene expression, enabling us to observe dissemination mechanisms in a controlled environment. We tested six kinases and phosphatases, Pp4-19C, Pez, CycT, CycD, Adk3, Alp-10, by employing RNAi knock-down to assess their roles in tumor cell dissemination. Dissections followed by confocal imaging of the midgut allowed us to visualize dissemination patterns. Our initial results indicate that several kinase and phosphatase pathways are essential for tumor cell dissemination, with their roles in cell cycle control, cell migration, proliferation, and nucleotide recycling. These findings provide a foundation for future studies to understand the specific pathways involved in cell morphology, motility, proliferation, and metabolism contributing to tumor cell dissemination.

258S **Mechanism of region-specific tumor-suppression in *Drosophila* epithelium** Tomonori Nakanishi[1], Masato Enomoto[1,2], Tomoe Kobayashi[3], Makoto Matsuyama[3], Tatsushi Igaki[1,2] [1]Graduate School of Pharmaceutical Sciences, Kyoto University, [2]Graduate School of Biostudies, Kyoto University, [3]Shigei Medical Research Institute

Cancer does not occur anywhere within a tissue. There are “tumor-susceptible” and “tumor-resistant” regions within a single tissue. In cancer research to date, many studies have focused on tumor-susceptible regions of tissues and why cancer is more likely to occur in those regions. On the other hand, few studies have focused on tumor-resistant regions and the regulatory mechanisms of these regions are still largely unknown. In *Drosophila* imaginal epithelium, oncogenic Ras-expressing cell clones with loss of apico-basal polarity gene *scrib* (Ras^{V12}/$scrib^{-/-}$) massively overgrow and exhibit invasive behavior. Using this malignant tumor model, we analyzed the spatial pattern of tumor-proliferative activities in *Drosophila* wing imaginal discs. We found that tumor growth of Ras^{V12}/$scrib^{-/-}$ clones was significantly suppressed in a specific tissue region called “notum”, while these clones aggressively overgrew in other parts of the wing epithelium. Interestingly, this region-specific tumor suppression was not observed when benign tumors with elevated EGFR/Ras signaling or inactivation of the Hippo pathway were induced in the wing discs. Our genetic data showed that the Upd-JAK-STAT signaling pathway essential for driving overgrowth and invasion of Ras^{V12}/$scrib^{-/-}$ malignant tumors, is downregulated specifically in the notum region. We are currently investigating the mechanism by which the notum region exerts a tumor-suppressive effect, which will be presented.

259S **Defining Molecular Mechanisms of Polymerase θ-Mediated Mitotic DSB Repair and Acentric Chromosome Segregation** Erin Dickert, Donald T Fox Pharmacology and Cancer Biology, Duke University

Cells must constantly protect their genome from DNA damage caused by endogenous and exogenous factors. DNA lesions in the form of DNA double-strand breaks (DSBs) are particularly deleterious if allowed to persist into mitosis. DSBs can generate acentric DNA fragments that lack canonical kinetochore-spindle attachments, posing risks for chromosome mis-segregation and micronuclei, aberrant nuclear structures linked to genome instability, and oncogenesis. However, how cells respond to mitotic DSBs remains poorly understood. We previously showed that *Drosophila melanogaster* hindgut papillar cells inactivate DNA damage response (DDR) checkpoints, like many cancers. Using this system, we identified a mechanism by which early-acting DNA repair factors are recruited to DSBs and resolved during mitosis. Repair foci resolution and downstream micronucleus prevention are dependent upon both monoubiquitination of the scaffold protein Fancd2 and the alternative end-joining (Alt-EJ) protein DNA polymerase θ (Polθ). Based on our findings, we propose a mechanism by which Alt-EJ displaces early-acting repair factors from and anneals broken DNA ends to form a "tether" by which acentric DNA segregates poleward. Our preliminary data demonstrate that both the helicase and polymerase functions of Polθ are required for papillar organogenesis upon DSB induction, suggesting that canonical Alt-EJ acts to repair mitotic DNA damage. Our current work aims to use a defined DSB system to sequence putative Alt-EJ events, and fluorescent tagging of Polθ during mitosis, to further understand Polθ mitotic function. These results will be discussed at the poster. Our ongoing studies will unveil a disease-relevant mechanism and novel regulators of acentric DNA segregation and mitotic DNA damage tolerance in DDR-attenuated cells.

260S **Identifying the functions of metazoan Nup98 and Nup96 during entry into S phase** Evi M Malagise, Jared T Nordman Biological Sciences, Vanderbilt University

Metazoans have thousands of sequence-independent DNA replication origins, which must be activated in a tightly-regulated manner to replicate the genome. DNA replication initiation begins when the origin recognition complex (ORC) binds to origins during G1 and recruits the replicative helicase and other replication factors. While the helicase is loaded in G1 phase, it is only activated in S phase. Despite our understanding of metazoan replication machinery, the factors that regulate ORC loading and helicase activation are not fully understood. We have found that replication initiation is affected by components of nuclear pore complexes (NPCs) in *D. melanogaster*. NPCs are large protein assemblies composed of ~30 unique proteins called nucleoporins (Nups) and are embedded throughout the nuclear envelopes of all eukaryotic cells. Interestingly, depletion of Nup98-96 prevents Drosophila cells from entering S phase without affecting the amount of chromatin-bound ORC2. We conducted an RNA-interference screen of all metazoan Nups and measured cell cycle progression by fluorescence activated cell sorting. We find that lack of entry into S phase is not generic to the NPC but rather specific to Nup98-96 and 3 other Nups. *Nup98-96* encodes a polypeptide that undergoes autoproteolysis and generates two proteins with distinct functions. Here, we investigate how Nup98 and Nup96 regulate DNA replication initiation and the entry into S phase.

261S **Identification of sumoylation targets of dTopors in testis in Drosophila melanogaster** Amel Moustafa[1], Deja Harris[1], Cynthia Douglass[1], Tasiyana Beza[1], Andrea M Binder[1], John Tomkiel Dean[2] [1]Biology, Univ North Carolina Greensboro, [2]Univ North Carolina Greensboro

The human tumor suppressor Topors (Topoisomerase I-interacting Arginine/Serine-rich protein) is a dual Ubiquitin/SUMO E3 ligase. The Drosophila homolog, dTopors, also acts as an E3 Ubiquitin Ligase. A role in sumoylation has not been demonstrated, however, it is suggested by homology to the human sumoylation domain, and in particular by the presence of a conserved sequence corresponding to a SIM (SUMO interacting motif) within this domain. In flies, *dtopors* mutants affect spermatocyte nuclear structure and disrupt chromosome segregation in male meiosis. We investigated the potential role of dTopors-mediated sumoylation in these processes. Using mass spectrometry of immunoprecipitated sumoylated proteins from wildtype versus *dtopors null* testis proteins, we identified putative targets of sumoylation by dTopors. Forty-three proteins were identified which were differentially sumoylated in wildtype vs. *dtopors*. Nine proteins were dependent on dTopors for sumoylation, whereas three were only sumoylated in the *dtopors* mutant. This suggests that dTopors acts as a SUMO ligase, and may also indirectly regulate other SUMO ligases. The largest gene ontology class amongst these differentially sumoylated proteins was chromatin and transcriptional control, but no candidates with known roles in nuclear structure or chromosome segregation were identified. We will present progress on using CRISPR-cas9 to generate a sumoylation-deficient separation-of-function mutant to query the requirement for sumoylation in chromosome segregation and nuclear structure *in vivo*.

262S **Deletion of Bloom Syndrome Helicase Regions Conserved Among Closely Related *Drosophila* Species Provides New Insights on Function** Evan B Dewey[1,2], Colleen C Bereda[3], Mohamed A Nasr[2], Venkat R Chirasani[4], Jeff Sekelsky[2] [1]Biology, Winthrop University, [2]Genetics, University of North Carolina at Chapel Hill, [3]University of North Carolina at Chapel Hill, [4]R.L. Juliano Structural Bioinformatics Core, University of North Carolina at Chapel Hill

Bloom Syndrome helicase (Blm) is a RecQ helicase with roles in homology-directed DNA double strand break repair (HDR), cell-cycle progression, and development. Pathogenic mutants in human *BLM* cause the autosomal recessive disorder Bloom Syndrome, with affected individuals predisposed to many different types of cancer. Previous work using *Drosophila Blm* mutants lacking helicase activity have shown defects in repairing DNA double-strand breaks (DSBs) by HDR, improper crossover patterning and segregation of chromosomes in meiosis, and compromised embryonic development due to cell-cycle progression defects. While Blm orthologs have a well conserved and structured RecQ helicase domain, much of the protein does not have a well characterized function and is poorly conserved across metazoa. Because of this, we compared closely related *Drosophila* species to identify regions of conservation that could suggest important functions. Two of these *Drosophila*-conserved regions in the N-terminus were deleted in *D. melanogaster* using CRISPR/Cas9 gene editing and Blm functions were then assessed. Deletion of either conserved region 1 (CR1) or conserved region 2 (CR2) compromised HDR in the synthesis-dependent strand annealing pathway and led to increased mitotic crossovers. CR2 was shown to be essential for embryonic development, but almost no effect was observed on this process when deleting CR1. CR1 deletion allows meiotic chromosomes to segregate properly, but does lead to defects in meiotic crossover designation and patterning. Last, we did not observe significant meiotic defects when CR2 was deleted. Thus, while the two regions have overlapping roles in Blm function, each also facilitates discrete Blm functions. These results provide novel insights into functions of the N-terminal region of Blm helicase and suggest important and distinct functional roles for previously uncharacterized Blm regions.

263S **Non-Uniform Chromosomal SNP Density Biases Sites of Meiotic Crossovers** Savanna Hinson[1], Ryan Sangston[2], Karol Cichewicz[3], Ishan Parikh[2], Jay Hirsh[2] [1]Biology, University of Virginia, [2]University of Virginia, [3]University of California, Davis

Dopamine serves dual functional roles in *Drosophila,* as a neurotransmitter in the central nervous system (CNS), and as a precursor for molecules required for cuticular hardening and pigmentation, the latter being crucial for fly viability. By selectively restoring cuticular dopamine synthesis in a dopamine-deficient background, we generated viable flies entirely lacking CNS dopamine. As expected, these flies initially showed reduced locomotor activity. Surprisingly, we identified a sub-line with normal activity levels despite a complete deficiency of CNS dopamine. To identify the genetic factor(s) responsible for this trait, we utilized a Genome-Wide Association Study (GWAS), mapping the trait to a roughly 3.5 mega-base (Mb) region of the X-chromosome. However, this mapping yielded ~5-fold lower resolution than anticipated, due to an uneven distribution of Single Nucleotide Polymorphisms (SNPs) between the two recombining lines. This biased recombination events towards regions of low SNP density, near but not within the the SNPs associating with the activity phenotype. Additionally, we observed crossover hotspots with a highly nonrandom distribution of crossovers within the SNP dense regions. These findings highlight challenges in *Drosophila* GWAS in situations where altered SNP density can skew recombination, complicating trait localization. Addressing these biases in recombination distribution is crucial for improving GWAS resolution in *Drosophila,* when the recombining strains contain non-uniformly distributed SNPs.

264S ***Drosophila* CRC Models to Study Tumor-Promoting Signaling Interactions** Brandon J Clark[1], Arushi Rai[1], Amit Singh[1,2,3,4], Madhuri Kango-Singh[1,2,3,4] [1]Biology, University of Dayton, [2]Premedical Programs, University of Dayton, [3]Center for Tissue Regeneration & Engineering (TREND), University of Dayton, [4]Integrative Science and Engineering (ISE), University of Dayton

Colorectal cancer (CRC) is the 2nd leading cause of cancer-related mortality in the US, with an estimated 53,000 deaths in 2024. Mutations in the dual tumor suppressor and proto-oncogene *p53*, the proto-oncogene *Ras*, and the tumor suppressor gene *APC* frequently co-occur in human CRC, underscoring its heterogeneity. The DNA damage repair pathway, mediated by the transcription factor p53, promotes cell cycle arrest and apoptosis in response to genotoxic stress. The Ras-MAPK pathway, regulated by the signal transduction protein Ras, triggers cellular proliferation and growth when active. The Wnt pathway, negatively regulated by Adenomatous Polyposis Coli (APC), likewise promotes cellular proliferation and growth through the activity of the transcription factor beta-catenin. The Hpo pathway and JNK pathway have also been found to crosstalk extensively with these pathways, regulating cellular proliferation, apoptosis, and growth. While the individual contributions of these signaling pathways in CRC have been well-documented, additional research is needed to better understand their interactions during tumorigenesis and tumor development. Thus, this study seeks to establish one-hit, two-hit, and three-hit models of CRC in *Drosophila* and to characterize them for cell cycle defects and altered cell signaling. To generate tumors, MARCM clones were made using *escargot-GAL4* to drive the expression of dominant-negative *p53*, oncogenic Ras^{G12V}, and/or loss-of-function APC specifically in intestinal stem cells in early larva/pupa and assessed in mature larvae and adults via dissection of third-instar larvae and immunohistochemistry. Here, we present preliminary data from these experiments and our progress in developing multi-hit preclinical models of CRC in *Drosophila*.

265S **Investigating the role of $CRL4^{Cdt2}$ and Condensin I subunits after DNA damage** Satya N Yalamanchi[1], Erin Dickert[1], Delisa E Clay[1], Daniel W Buster[2], Gregory C Rogers[2], Donald T Fox[1] [1]Pharmacology and Cancer Biology, Duke University, [2]Cellular and Molecular Medicine, University of Arizona

The segregation of acentric DNA resulting from double stranded breaks (DSBs) that persist into mitosis is crucial to preventing the formation of micronuclei, which can lead to catastrophic genome shattering known as chromothripsis and overall genomic instability. Previously, we have shown that the rectal papillar cells in Drosophila are able to segregate acentric DNA fragments during mitosis despite inactivating interphase DNA damage checkpoint responses. Using this model, we found the cullin-RING ubiquitin ligase 4 complex ($CRL4^{Cdt2}$) to be a crucial regulator in responding to mitotic DSBs. To uncover possible targets of $CRL4^{Cdt2}$ that act in the context of DNA damage, we immunoprecipitated Cdt2-bound proteins that are specific to cells with DNA damage. From these efforts, we identified two subunits of the Condensin I complex, CAP-D2 and CAP-G, as potential DNA damage-specific substrates of $CRL4^{Cdt2}$. Both subunits contain PCNA-interacting protein domains (PIP boxes) that enable their interaction with $CRL4^{Cdt2}$, potentially implicating them in the segregation of acentric DNA into daughter nuclei. In order to better understand how $CRL4^{Cdt2}$ regulates these Condensin I subunits, we have employed two approaches: (1) knockdown of the $CRL4^{Cdt2}$ complex and (2) deletion of the PIP box in CAP-D2. Understanding the role of CRL^{Cdt2} and Condensin I subunits in preventing the formation of micronuclei and elucidating the pathway by which cells attenuate the effects of mitotic DSBs can improve our understanding of cancer and identify novel therapeutic targets.

266S **Dissecting Fox transcription factor-mediated regulation of Polo kinase activity essential for cardiac progenitor cell divisions** Rajnandani Katariya[1,2,3], Abbigayle J Gamble[1,2], M. Rezaul Hasan[1,2,3], Kuncha Shashidhar[1,2,3], Mofazzal K Sabbir[1,2,3], Shaad M Ahmad[1,2,3] [1]Department of Biology, Indiana State University, [2]The Center for Genomic Advocacy, Indiana State University, [3]Rich and Robin Porter Cancer Research Center, Indiana State University

Forkhead box (Fox) transcription factors play a crucial role in heart development (cardiogenesis) in both mammals and Drosophila. Previous research from our laboratory has demonstrated that the Drosophila Fox gene *jumeau* (*jumu*) mediates three distinct categories of cardiac progenitor cell division—asymmetric, symmetric, and cell division at an earlier developmental stage—by upregulating Polo kinase activity. However, when we compared the transcriptional expression profiles of flow cytometry-sorted mesodermal cells from wild-type embryos and *jumu* null mutants, we found that *jumu* does not transcriptionally regulate *polo*. This result suggests that that the Fox-mediated activation of *polo* in cardiac progenitor cell division may occur posttranscriptionally. A vital step in the posttranscriptional activation of polo is the posttranslational phosphorylation of the Polo protein. Two serine-threonine kinases that activate Polo via phosphorylation are Aurora B (AurB) and Back Seat Driver (BSD). Intriguingly, both *aurB* and *bsd*, the genes encoding these kinases, are transcriptionally activated by *jumu*, suggesting that they may be promising candidates through which the Fox gene may activate *polo*. While *aurB* is known to have multiple roles in mitosis, bsdhas previously been characterized only as mediating the postmitotic activation of Polo necessary for somatic muscle morphogenesis. However, this did not rule out the possibility that bsd is also utilized for mitosis in cardiac progenitor cells. If *jumu* uses *aurB* and *bsd* to activate polo for cardiac progenitor cell division, then we would expect *aurB* and *bsd* mutants to exhibit cardiac defects similar to those seen in *jumu* and polomutants. In this study, we show that loss-of-function mutations in *aurB* and *bsd* do, in fact, reproduce all three categories of cardiac progenitor cell division defects observed in *jumu* and *polo* mutants, consistent with our hypothesis.

We are currently conducting genetic interaction and rescue assays to definitively determine whether *aurB* and *bsd* mediate Polo activation by *jumu*. If *jumu* does indeed activate Polo using one of these candidate genes, then that candidate gene should exhibit pairwise synergistic genetic interactions with both *jumu* and *polo*. Furthermore, cardiac-specific ectopic expression of that candidate gene should also partially rescue the cardiac progenitor cell division defects seen in *jumu* mutants, but not in *polo* mutants.

267T **Decoding Caspase Substrate Cleavage: New Insights from *Drosophila* Proteomics** POOJA RAI[1], Erik Gomez Cardona[2], Jainilkumar Gomez Patel[2], Olivier Gomez Julien[2], Andreas Bergmann[1] [1]MCCB, University of Massachusetts Medical School, [2]Department of Biochemistry, University of Alberta

Caspases, a pivotal family of 7 cysteine proteases in *Drosophila* (12 in humans), orchestrate critical cellular processes, including programmed cell death (apoptosis) and non-apoptotic roles such as proliferation, cellular migration, pruning, innate immunity and others. These proteases exhibit a strong substrate preference for aspartate residues, catalyzing cleavage events that drive hallmark morphological changes in apoptotic cells like membrane blebbing, DNA fragmentation, and phosphatidylserine externalization. Recent advancements in proteomics have revolutionized the identification of caspase substrates, elucidating their broader functional repertoire beyond apoptosis.

In this study, we employed forward N-terminomics coupled with Subtiligase enzymatic labeling and LC-MS analysis to systematically map caspase cleavage events in apoptotic cell lysates. Our analysis identified 81 substrate cleavage sites (P1 aspartate) in 60 proteins, implicating proteins involved in translation, metabolism, and neuromuscular growth pathways. These insights advance our understanding of caspase-mediated proteolysis in apoptosis and cellular remodeling processes.

268T **Single cell transcriptomics reveal distinct cell clusters regulating Apoptosis-induced Proliferation** Prathibha Yarikipati, Andreas Bergmann Molecular Cell and Cancer Biology, University of Massachusetts Chan Medical School

Apoptosis-induced Proliferation (AiP) is a mechanism by which apoptotic cells release growth factors that stimulate neighboring cells to divide. We study AiP by co-expression of the pro-apoptotic factor *hid* with the baculoviral gene *p35*, which blocks effector caspase activation and promotes Dronc-mediated AiP. When expressed under *ey-Gal4* control, this combination triggers overgrowth of adult fly heads which is established by expansion of the *ey*-expressing region at the expense of the photoreceptor region in early imaginal discs. Here, to uncover the transcriptional dynamics underlying this overgrowth, we performed single-cell RNA sequencing (scRNA-seq) of larval eye imaginal discs from the AiP model (*ey>hid*; *p35*) and its controls (*ey>p35* and *ey>WT*). Our analysis revealed distinct hid-expressing clusters with unique transcriptional profiles in overgrown eye discs that were absent in the controls. Immunostainings showed that cells adjacent to photoreceptor region are highly enriched for P35, Hid and MMP1 (a marker for JNK which is required for AiP), indicating that these spatially organized clusters play an important role in AiP. These *hid*-expressing clusters were surrounded by proliferating cells and showed no evidence of senescence, contrary to previous reports. We did not find any significant differences in transcriptional profile of photoreceptor clusters, but their cell numbers were reduced due to the overgrowth of adjacent cells. In addition, we also observed elevated expression of metabolic factors including mitochondrial, ER- and Golgi-related genes in eye imaginal discs that are enhancing the growth of these regions in the overgrown eye discs. This scRNA-seq analysis reveals an unexpected degree of *hid/p35*-induced responses during overgrowth and provides a specific transcriptional framework for understanding AiP responses in *Drosophila*.

269T **Investigating cell signaling genes controlling the clearance of dying cells in the ovary.** Jeanne Peterson, Diane V. Lebo, Kim McCall Biology, Boston Univ

During the final stage of oogenesis, somatic follicle cells within the ovary clear away the nurse cell nuclei. In a screen of kinase genes, the knock-down of the G-protein coupled receptor kinase-2 (*Gprk2*) produced a phenotype. The final stage egg chambers showed defects in engulfment visible as persisting nurse cell nuclei. Double knockdown analysis indicates that *Gprk2* in the *Ced 12* pathway acts in parallel to *Draper*. In addition, the Dumpless phenotype indicates that *Gprk2* might act in an additional pathway.

270T **Pink1 represses apoptosis and allows proper morphogenesis after exposure to ionizing radiation in *Drosophila melanogaster*** Lauren Orr, Hannah Golding, Elizabeth A O›Brian, Joshua Siauw, Tin Tin Su University of Colorado

Autophagy has emerged as a potential target for enhancing the efficacy of radiotherapy but its precise role in the cellular response to ionizing radiation (IR) remains enigmatic. To address this, we conducted a focused RNA interference (RNAi) screen in Drosophila melanogaster targeting key autophagy regulators. Using adult compound eye morphology as a readout, we identified two genes of interest, Pink1 and ref(2)P (p62/SQSTM1), both essential for mitophagy, a specialized form of autophagy responsible for the selective degradation of damaged mitochondria. Knockdown of these genes in larval eye discs resulted in adult eye defects when the larvae were irradiated during the 3rd instar stage. Cell biological analyses revealed that Pink1 RNAi expression did not affect cell proliferation or double-strand break (DSB) recognition but significantly increased apoptosis following IR exposure. This finding was consistent in loss-of-function mutants of both Pink1 and its partner Parkin. Increased apoptotic activity after Pink1 depletion was notably concentrated in the G1-arrested morphogenetic furrow (MF), as confirmed by co-staining with cDcp-1 and Cyclin B. However, the protective role of Pink1 was not cell cycle stage–specific, as arresting other cells of the eye disc in G1 did not alter their propensity to undergo IR-induced apoptosis upon Pink1 knockout. The MF is characterized by elevated Dpp (TGF-β) signaling. Current studies are dedicated to determining if mitophagy is specifically required in cells with high Dpp signaling for mitigating IR-induced apoptosis and ensuring proper eye morphogenesis during recovery from radiation-induced damage.

271T **Molecular mechanisms in cell competition and ribosomopathy** Mallory Stephens[1], Olivia Monet Gramza[2], Neha Joshi[3], Chelsea Nguyen[4], Chaitali Khan[5], Harrison Hector[6], Nicholas E Baker[7] [1]Microbiology and Molecular Genetics, University of California at Irvine, [2]Developmental and Cell Biology, University of California at irvine, [3]Micorbiology and Molecular Genetics, University of California at Irvine, [4]Mathematical, Computational, and Systems Biology Program, University of California at Irvine, [5]Cell and Developmental Biology Center, NHLBI, NIH, [6]Genetics, Albert Einstein College of Medicine, [7]Microbiology and Molecular Genetics, Developmental and Cell Biology, University of California at Irvine

Cell competition is a process whereby certain cells are lost and replaced from developing tissues if fitter cells are available to replace them. The classic example is that of 'Minute' cells, heterozygous for Ribosomal protein gene mutations, which are eliminated from genetically mosaic imaginal discs that also contain wild-type cells. This may be an adaptation to eliminate aneuploid cells, because the genome-wide distribution of Rp genes leads to Rp gene haploinsufficiency in many aneuploid genotypes. Cell competition, as well as multiple cell-autonomous properties of Minute cells, are controlled transcriptionally by the bZip domain protein Xrp1. Genes differentially expressed in Minute cells in an Xrp1-dependent manner are being tested for their contributions to cell competition and to cell-autonomous consequences of Rp gene haploinsufficiency using a FLP/FRT-based method to generate aneuploid cells. Meanwhile, mRNAs translated differentially in Minute cells are being identified by ribosome profiling of wing imaginal discs. It will be interesting to assess the possible tumor suppressor function of cell competition when applied to aneuploid cells in mammals, whether it might be enhanced to reduce cancer prevalence, and how it fails, so allowing aneuploid cells to continue developing toward cancer.

272T **UFMylated ER Proteins Scaffold p62 Condensates for Regulation of Neuronal Structural Plasticity** Shashank Shekhar[1], Charles Tracy[2], Gouxue Wang[3], Helmut Krämer[3] [1]UT Southwestern Medical center, [2]UT Southwestern MEdical Center, [3]UT Southwestern Medical Center

Structural plasticity of photoreceptor neurons relies on endoplasmic reticulum (ER) quality control. One recently emerged aspect of ER quality controls is the modification of ER proteins by the Ubiquitin-like UFM1 protein. However, the mechanisms by which this UFMylation regulates neuronal structural plasticity is still a mystery. We utilized *Drosophila* photoreceptors as a model system and a constant ambient light (LL) paradigm as a stressor to induce structural adaptation. This adaptive change involves multiple stress response pathways including the unfolded protein response in the ER (UPR^{ER}) and is reversible upon stress removal with return to a 12h light 12h dark cycle (LD).

Under LL, we observed fragmentation of the ER and a significant increase in UFM1 puncta. Notably, we discovered that the Ref(2)p/p62 (onwards p62) adaptor protein undergoes liquid-phase separation to form condensates in photoreceptor neurons under LL. The formation of p62 condensates depended on photoreceptor activity and on eIF2α-S50 phosphorylation, a hallmark of the integrated stress response.

To investigate the role of UFMylation in adaptation, we employed CRISPR-Cas9 to endogenously tag UFM1 with an Alfa epitope, followed by UFMylated proteome enrichment and mass spectrometry. PTM analysis of UFMylation enriched proteome from fly heads identified several ER resident UFMylated proteins including Homer, Serca, and IP3R. High-resolution Airyscan images after LL show p62 condensates decorated with ER membrane fragments, visualized by Homer and Calnexin, and partially colocalized with UFM1 puncta. p62 condensates are abolished when UFMylation in photoreceptors is blocked by RNAi against UFM1 or Uba5, its non-canonical E1 ligase. Western blots show accumulation of UFMylated proteins in p62 null mutants indicating role of p62 in the clearance of UFMylated substrates. Moreover, loss p62 or its binding to ATG8 in the p62[LiR] allele or loss of UFMylation resulted in disorganized rhabdomeres and reduced postsynaptic responses under LL conditions. Interestingly, in the p62[LIR] allele, Rh1 rhodopsin accumulated even under LD, indicating that p62-dependent autophagy plays a crucial role in Rh1 recycling.

In summary, we uncovered a novel in-vivo function of p62 condensates in neuronal structural plasticity through UFMylation of ER proteins. ER stress triggers the UFMylation, providing a scaffold for p62 droplet formation, which is vital for neurons to adapt to environmental changes.

273T **Defective cell clearance in perturbed *Drosophila melanogaster* lines** Pamela Yang, Guangmei Liu, Trung Le, Cheng Yang Shi, Jerald Shin, Kim McCall Boston University

The *draper (drpr)* gene in *Drosophila melanogaster* encodes for a phagocytic receptor critical for cell clearance by maintaining multiple tissues through clearance of dead cells, bacteria and debris. Previous research has determined that in addition to playing an essential role in the brain where mutations can cause neurological problems, the phagocytic removal of nurse cells in the *Drosophila* ovary by follicle cells is dependent on *drpr.* Disruptions of the gene cause the accumulation of nurse cell nuclei in late-stage egg chambers because the clearance by stretch follicle cells no longer proceeds normally. Since the currently used $drpr^{\Delta 5}$ line targets the noncoding region, mutant alleles with a deletion of the *drpr* coding region using CRISPR/Cas9 technology was attempted to ensure that gene function is completely disrupted. Molecular analysis revealed a number of lines with small deletions, and one line (CR1) with a deletion of 1107 base pairs, removing all of exon 5 and 6 which encode a large section of the extracellular domain. Flies homozygous for this allele show defective cell clearance similar to $drpr^{\Delta 5}$ where large quantities of persisting nurse cell nuclei (pnc) were still present in stage 14 egg chambers. Antibody staining revealed a strong decrease in Draper protein expression in ovaries of the CR1 line. In addition to examining mutant *draper* alleles, comparative analysis was undertaken with tissue-specific RNA interference approaches to knock down *draper*. Follicle cell *tj-Gal4* and *GR1-Gal4* lines were used to systematically target *drpr* with two different $drpr^{RNAi}$ lines: a short-hairpin (Val20) and a long hairpin. Comparisons showed a strong increase in pncs for tjGal4 > drprRNAi Val20 crosses. Expanding on the RNAi approach, two other binary systems, LexA and Q, were investigated. Phenotypic results are consistent with *drpr* dysregulation showing large quantities of pncs present in late-stage egg chambers and a strong decrease in Draper protein expression. In addition to the ovary, we are comparing the strengths of different *draper* mutants and knockdowns in the adult brain. Knocking down *draper* with the different binary systems provides the ability to control gene expression in two different tissues within the same fly by combining the Gal4-UAS with a second binary expression system. Comparisons of the different knockdowns will be presented.

274F **The integrated stress response transcription factor ATF4 regulates border cell collective motility** Rehan Khan, Emily Burghardt, Jocelyn McDonald Division of Biology, Kansas State University

Many cells move as collectives during development and in diseases like cancer, but the mechanisms are poorly understood. *Drosophila* border cells represent an excellent model for investigating how cell collectives migrate in vivo. In the ovary, 6-10 follicle cells form the border cell cluster, which moves collectively between large nurse cells towards the oocyte. Migrating cells, especially those that migrate collectively in crowded tissues, experience physiological and mechanical stresses. One stress encountered by cells is an increase in misfolded proteins, which can cause endoplasmic reticulum (ER) stress. The integrated stress response (ISR) is a major pathway that helps cells recover from ER stress. We recently found that the ISR pathway is required and active in border cells, indicated that border cells experience ER stress that needs to be counteracted during normal migration. A vital component of the ER stress response is the transmembrane sensor kinase PERK, which phosphorylates eIF2α, thus blocking global mRNA translation. Notably, activation of PERK also selectively induces the translation of a small number of mRNAs crucial for cellular recovery, including the bZIP transcription factor ATF4. How ATF4 mediates collective border cell migration via stress recovery is unknown. Here, we show that both overexpression and knockdown of ATF4 results in significant migration defects, indicating that a balance of ATF4 activity is critical. We found that a known target of ATF4, the eIF4E-binding protein (4E-BP; Thor) is expressed in migrating border cells in an ATF4-dependent manner. Further, we find that delta-glutathione-S-transferase-GFP (*gstD*-GFP), a reporter of oxidative and proteotoxic stress, is endogenously expressed in border cells. Mutation of a ATF4 binding site increases the levels of gstD-GFP, suggesting that ATF4 mediates expression of gstD genes. At the cellular level, optimal levels of ATF4 are critical for border cell motility. ATF4 overexpression inhibits the ability of live border cells to delaminate from the follicle cell epithelium. In clusters with a mix of wild-type and ATF4 overexpressing cells, border cells with high ATF4 sort to the back, indicating compromised motility. Knockdown of ATF4 also impairs migration, though to a lesser extent than ATF4 overexpression. These results together demonstrate that border cells require an active ATF4-dependent stress response program to delaminate and migrate successfully as a collective.

275F **Subcellular Mechanisms of Programmed Cell Death During Drosophila Oogenesis** Georgette-Vanelle Wandji, Ten Harder, Kimberly McCall Boston University

The processes of cell death and clearance of cellular debris are essential to the development and homeostasis maintenance of an organism. These phenomena in *Drosophila* oogenesis play critical roles in ensuring the proper development of the female reproductive system and the formation of functional eggs. In the *Drosophila* ovary, germline-derived nurse cells (NCs) undergo non-apoptotic programmed cell death as part of normal development. We have found that NC death is controlled largely non-autonomously by the surrounding somatic stretch follicle cells (FC), and that the phagocytic machinery is required for the final steps of NC death: acidification, nuclear membrane breakdown, and DNA fragmentation. Lysosomal machinery acting in the surrounding FCs non-autonomously promotes the acidification and DNA fragmentation of nurse cells. To gain a comprehensive view of how the stretch follicle cells carry out NC death non-autonomously, we have examined how the cells' endoplasmic reticulum and plasma membrane are eliminated. We find that the NC membrane begins to break down prior to acidification and we hypothesize that the NC plasma membrane undergoes trogocytosis by the neighboring FCs. In addition, Draper, a phagocytic protein found in FCs is necessary for the proper engulfment of NCs. We showed that NC membrane dismantlement and removal is dependent on this engulfment gene. By studying the interactions between the plasma membranes of FCs and NCs during the late stages of oogenesis, we aim to gain deeper insights into the mechanisms of NC death and clearance. Furthermore, we will explore the dynamic relationship between vesicles associated with FCs and the NC plasma membrane during this process. This work enhances our understanding on mechanisms of phagocyte-dependent cell death.

276F **The Roles of CASK and Calcium Signaling in Apoptosis-induced Proliferation** Daniela M Dominguez[1], Md Iqramul Haque[2], Yun Fan[1] [1]School of Biosciences, University of Birmingham, [2]Department of Physiology, Bangladesh Agricultural University

Apoptosis is a well-conserved, programmed form of cell death. Key players of this process are caspases, or cysteine aspartic proteases, which also have functions outside of cell death. One such function is in driving apoptosis-induced proliferation (AiP), a compensatory mechanism during which stress-induced, dying cells emit signals to trigger proliferation of their neighbours. AiP is important in aiding the replacement of lost cells to promote tissue regeneration, and as part of well-functioning tissue homeostasis. However, aberrations in AiP can contribute to tumorigenesis and neurodegeneration. Therefore, understanding AiP regulation has important implications for human disease. Using *Drosophila* as a model organism, we have recently demonstrated that actin cytoskeleton remodelling is critical for AiP, ultimately triggering the stress-response c-Jun N-terminal kinase (JNK) signaling to induce tissue proliferation. However, the regulation of actin dynamics and our understanding of mechanisms which underpin AiP require further elucidation. Interestingly, we have identified calcium/calmodulin dependent serine protein kinase (CASK), an evolutionarily conserved scaffolding protein, as a potential regulator of actin dynamics in AiP. This reveals a novel function for CASK outside of its known roles in neurodevelopment. CASK is known to interact with calcium/calmodulin-dependent protein kinase II (CaMKII), a key transducer of calcium signaling. We provide evidence that CaMKII, calcium signaling, and potential interactions with CASK play critical roles in regulating AiP. Our findings offer new insights into the underlying mechanisms driving this phenomenon.

277F **Invitro Exploration of the Effects of Methanolic Plant Extracts on the Mycelial Inhibition of Corynespora cassiicola of Rubber Leaves (Hevea brasiliensis)** Benjamin Ewanole Ohiocheoya[1], Nicholas Obehi Ogbebor[2,2], Danjuma Salisu Ibrahim[1] [1]Crop Protection, Rubber Research Institute of Nigeria, [2]Information and Documentation, Rubber Research Institute of Nigeria

Hevea brasiliensis is affected by a myraid of leaf diseases caused by pathogens that are both microscopic and visible parasites as well as by others of non-pathogenic origin. Rubber tree when infected by pathogens that affects the folial portion causes devastating effects especially at their developmental stages in the nursery, but also on the young and adult trees. Chief amongst these pathogens that infects the Hevea tree especially in Nigeria is the fungi - Corynespora casiicola. C. casiicola causes the fall of both young and old leaves all year round. This study was carried out to evaluate the effect of Plant extracts on the mycelial growth inhibition of Corynespora casiicola of rubber leaf (Heveabrasiliensis). The fungal isolate was isolated from naturally diseased leaf of Hevea brasiliensis collected from Rubber Research Institute of Nigeria's plantation in Iyanomo, Benin city. This isolate was then identified, culturally, morphologically and microscopically. Ten plant leaves were extracted using crude method and the extract was tested against C. Casiicola to monitor the effect on conidial germination and mycelial growth. The concentrations of the extracts were varied and tested against C. casiicola using the poison-food technique. The data generated during the study were subjected to various descriptive and inferential statistics. Of the ten plant extracts used in the study, only four (Allium sativum, Ageratum cornyzoides, Amaranthus viridis and Ocimum basilicum) were found to have significant antifungal effect against the pathogen. Extract from Fiscus exasperata (one of the plant without any inhibitory effect) was found to further promote the growth of C. casiicola without any inhibitory effect on the pathogen. The mycelia inhibition of the four active plant extracts revealed a better antifungal effect at 100% for O. basilicum and A. sativum. The minimum inhibitory concentration of the plant extract against the tested pathogen showed A. viridis (32%) and O. basilicum (33%). The percentage of mycelia inhibition of fungicides and active plant extracts revealed a favourable comparison with the usage of commercial fungicides and as result plant extracts can serve as a viable alternative to the use of chemical fungicides.

278F **Social Modulation of Autophagy in the *Drosophila* Brain** Marta Rozados Barreiro, Priyanka Mittal, Pelin Cayirlioglu Volkan Duke University

Social experiences play a crucial role in brain health, influencing neural circuits and stress pathways affecting cellular processes such as autophagy. Autophagy is a critical metabolic process that enables cells to respond to stress by breaking down and recycling unnecessary or dysfunctional components, such as misfolded proteins and damaged organelles. We investigated transcriptional profiles from *Drosophila melanogaster* male brains in wild-type (CS). We found that social isolation (SH) led to downregulating autophagy-related gene expression compared to group-housed (GH) conditions. Analysis of pheromone receptor mutants, *Or47b* and *Or67d*, along with the transcriptional regulators *doublesex* (*dsx*) and *fruitless* (*fru*), revealed distinct roles in modulating autophagic gene expression in response to social context. In socially isolated (SH) brains, *Or47b* neural circuits and *dsx* function drive transcriptional responses, as mutations in these genes lead to profiles resembling those of group-housed (GH) brains. Conversely, in GH conditions, *Or67d* neural circuits and *fru* function alter transcriptional responses, with mutations causing profiles more closely resembling those in isolated brains. These findings suggest that the two pheromone circuits, along with Dsx and Fru, act antagonistically, where *Or47b*/Dsx promote transcriptional changes in response to isolation and *Or67d*/Fru drive responses to group housing. Additionally, we investigated the effects of social isolation on autophagy in *the Drosophila* brain using the autophagic marker Atg8a-mCherry. We observed social isolation reduced autophagy in brain regions associated with sensory processing and neuroendocrine signaling, compared to group-housed flies. Interestingly, we also noticed increased autophagy levels in group-housed flies in the morning versus evening, while isolated flies showed no circadian variation. These findings suggest that social experiences significantly influence autophagy in the Drosophila brain, likely through interactions between pheromone signaling pathways and transcriptional regulators of social behavior. The differential regulation of autophagy-related genes in response to social context, combined with circadian modulation of autophagic activity observed only in group-housed flies, underscores the complexity of autophagy regulation in response to social and circadian cues.

279F **4EHP and NELF-E regulate physiological ATF4 induction, amino acid metabolism, and proteostasis in disease models** Kristoffer Walsh[1,1], Hidetaka Katow[1], Hannah Junn[1], Deepika Vasudevan[2], Christoph Dieterich[3], Hyung Don Ryoo[1] [1]Cell Biology, NYU Grossman School of Medicine, [2]Cell Biology, University of Pittsburg School of Medicine, [3]Internal Medicine III, University Hospital Heidelberg

Cells adapt to proteostatic and metabolic stresses, in part, by initiating homeostatic signaling pathways. One such pathway, referred to as the Integrated Stress Response (ISR), is initiated by stress-activated kinases that phosphorylate the translation initiation factor, eIF2a. Reflecting the broad role of ISR in responding to diverse cellular stress, there is an increasing list of metabolic and degenerative diseases associated with abnormal ISR signaling. The ISR involves an intriguing combination of translational control: While stress-induced eIF2a phosphorylation attenuates global mRNA translation, it also causes a preferential translation of ATF4. Once induced, ATF4 stimulates the expression of various proteostasis and amino acid biosynthetic genes. ATF4's preferential induction requires the regulatory elements in ATF4 mRNA's 5' leader that contains upstream Open Reading Frames (uORFs). uORFs generally reduce downstream ORF translation due to translational termination at the uORF stop codon, but ATF4's uORF helps to promote ATF4 synthesis through a poorly understood mechanism. Here, we report a previously unrecognized pathway that regulates ATF4 expression during ISR signaling. Specifically, we found *4EHP* as a gene required for *Drosophila* ATF4 (also *crc*) induction in the larval fat body and in disease models associated with abnormal proteostasis. Consistent with ATF4's known role in metabolism, the loss of *4EHP* reduced the expression of well-established ATF4 target genes. *4EHP* encodes a poorly understood mRNA cap-binding protein, and we used TRIBE (Targets of RNA-binding proteins Identified By Editing) to profile interacting mRNAs. Among those that were specifically bound to 4EHP's cap-binding domain was *NELF-E* mRNA*,* which encodes a regulator of pol II-mediated transcription. Both *NELF-E* and *4EHP* were required for the expression of multiple eIF3 subunits. One was *eif3h,* a factor implicated in the translation of transcripts with uORFs. We found that reduction of *eif3h* did not affect control reporter expression, but specifically suppressed ATF4 expression. *eif3h* reduction also inhibited a reporter for ATF4's 5' regulatory reader sequence. These results uncover an ATF4 regulatory network involving *4EHP, NELF-E*, and *eIF3h,* impacting gene expression control by the ATF4 5' leader.

280F **EMS mutagenesis screen to explore mechanisms of the newly identified cell death, erebosis in the *Drosophila* midgut** RAHUL PARIT, Sa Kan Yoo Laboratory for Homeodynamics, RIKEN Center for Biosystems Dynamics Research (BDR)

In the *Drosophila* midgut, enterocytes play a critical role in nutrient absorption and maintaining the gut barrier integrity. These cells are continually renewed through homeostatic turnover, as stem cells differentiate to produce new enterocytes. We have recently identified a novel form of enterocyte death, distinct from conventional pathways, which we have named erebosis. Erebotic cells are characterized by the accumulation of Angiotensin-converting enzyme (Ance) and the loss of various proteins. Erebotic cells exhibit the loss of key cellular components including organelles, cytoskeleton, and cell adhesion, emphasizing the unique nature of this cell death event. Despite these observations, the molecular mechanisms underlying erebosis remain unclear. In this study, we are performing EMS mutagenesis as a genetic screening approach to identify key genes that suppress erebosis, with a focus on the X chromosome in hemizygous males. We have identified a mutant that demonstrates suppression of erebosis in the midgut. We are in the process of functionally characterizing this mutant and identifying the responsible gene through whole-genome sequencing. We will discuss our current progress in genetic screening aiming at discovering mechanisms of erebosis.

Keywords: Erebosis, *Drosophila* midgut, enterocytes, EMS screening, X-chromosome

281S **Unraveling the Impact of Dominant Activating Rac Mutations on Cellular Behaviors: Insights from Drosophila Border Cells** Morgan S Smith[1], Abhinava Mishra[1], Denise J Montell[2] [1]MCDB, University of California, Santa Barbara, [2]MCBD, University of California, Santa Barbara

Ras-related C3 botulinum toxin substrate 2 (Rac2) is a small signaling GTPase crucial for proper immune system development and function. It cycles between GTP-bound (active) and GDP-bound (inactive) states, regulated by guanine nucleotide exchange factors (GEFs) and GTPase-activating proteins (GAPs). There are **15 different dominant activating mutations in Rac2 found in human patients who have a spectrum immunodeficiencies from T- and B- cell lymphopenia to severe bone marrow hypoplasia.** However, the underlying mechanisms by which activated Rac2 causes immunodeficiencies remain unknown. Rac proteins are highly conserved in sequence and function from flies to humans, and key insights into possible mechanisms have recently come from studies of border cells in the *Drosophila melanogaster* egg chamber. Our lab found that border cells expressing constitutively active RacG12V engulf and kill the neighboring nurse cells, resulting in egg chamber destruction. Thus hyperactive Rac is sufficient to cause one cell to eat and kill another living cell *in vivo*. Intriguingly, our lab further found that hyperactivating Rac2 mutations also cause mouse and human macrophages to engulf and kill T cells, offering a potential explanation for the disappearance of circulating T cells observed in Rac2+/E62K patients and mice. Building on this work, here we use Drosophila **border cells** as a model to express **different,** dominant activating Rac2 mutations found in human patients and assess the cell autonomous and cell non-autonomous effects of hyperactive Rac on motility, phagocytosis, and tissue destruction. **My data show that Rac mutants have distinct effects on migration and cannibalistic behavior of border cells.** In the future, we plan to test effects of Rac mutations on Drosophila hematopoiesis, which takes place in the lymph gland and has recently emerged as a powerful model for blood cell development and function. Leveraging the egg chamber and lymph gland models will improve our understanding of the effects of Rac mutations on cell behaviors in vivo and contribute to elucidating the pathogenesis of human hematopoietic disorders.

282S **Elucidating the physiological function of evolutionarily conserved heat shock protein 110 in Drosophila** Beatriz Rios[1], Shiyu Xu[1], Stephen M Farmer[1], Unekwu M Yakuba[2], Xin Ye[1], Daniela Covarrubias[3], Kevin A Morano[2], Sheng Zhang[1] [1]Center for Metabolic and Degenerative Diseases, University of Texas Health Science Center at Houston, [2]Microbiology and Molecular Genetics, University of Texas Health Science Center at Houston, [3]Biosciences, Rice University

As a key hallmark of neurodegenerative disease, misfolded proteins and their resulting aggregates are of great interest. Chaperone proteins and physiologically essential substrates work in conjunction to establish and maintain the correct protein conformation, preserving protein homeostasis and overall cellular health. Previous research has firmly established the importance of heat shock proteins (HSPs) as chaperones and extensively delineated their biochemical functionality. *In vitro* experiments established HSP110 as a powerful "holdase", when compared to other heat shock proteins, allowing it to sequester misfolded proteins but lacking the ability to refold them. Although HSP110's biochemical role in the disaggregation machinery has been greatly explored through *in vitro* experiments and the unicellular eukaryote *Saccharomyces cerevisiae*, the characterization of its physiological functionality in higher eukaryotes is limited and requires further investigation, especially in postmitotic neurons that are non-renewable and require robust cellular homeostasis activity to maintain their longevity and function. *Drosophila* is an ideal model organism because the fly genome is amenable for easy genetic manipulation to elucidate mechanistic details of protein function. Also, there exists only a single HSP110 gene (dHsp110) in *Drosophila* genome as compared to three HSP110-like genes in both mouse and human genomes, therefore greatly simplifying its functional study. To facilitate the detection and manipulation of endogenous dHsp110 protein, we characterized and validated two genome trap lines. Using a hybrid *pigP* element, an artificial exon was introduced within the open reading frame of Hsp110, using a splicing acceptor and donor flanking and artificial open reading frame encoding a chimera flag-strep-venusYFP-strep (FSVS) tag, so it becomes part of the coding region and is transcribed with the native dHsp110. The tagged dHsp110 protein therefore can then be visualized through the inserted Venus-YFP tag. Importantly, in these two genome tagging lines, all dHsp110 proteins are expressed as dHsp110-FSVS fusion and the inserted chimera tag does not interfere with the normal function of dHsp110 protein. Examining larvae containing the FSVS genome trap line, we found dHsp110 was ubiquitously expressed in neuronal tissue and had a cytoplasmic localization. Leveraging this tool, future work will explore the functional consequence of gain and loss of Hsp110 function, specifically in neurons, and the chaperone's function in the clearance mechanisms employed to remove protein aggregates in neuronal tissues in models of different neurodegenerative disorders such as Alzheimer's and Parkinson's diseases.

283S **The Role of Hemocytes in Regeneration Following Necrotic Ablation** Maksym Dankovskyy, Robin Harris Arizona State University

Apoptotic cells are known to communicate to adjacent cells via the release of mitogens that encourage cell proliferation and survival, a process generally known as apoptosis-induced proliferation (AiP). Studies of this phenomenon have, in part, relied on the undead cell model in which cells over-express pro-apoptotic factors but are unable to die due to p35 inhibition of effector caspases. This model has revealed important relationships between non-apoptotic functions of the initiator caspase Dronc, reactive oxygen species, basement membrane (BM) disruptions, and hemocytes, all of which are necessary to sustain tumorigenesis in the undead model. However, traditional apoptotic genetic ablation systems that injure wing imaginal discs fail to disrupt the BM. As a result, regeneration following such ablation seems to occur without hemocyte signaling.

In the case of necrosis, an unregulated form of cell death characterized by membrane swelling and leakage, comparatively little is known about its effects on adjacent cells. Our lab has pioneered the study of this in *Drosophila* by developing two novel necrotic ablation systems. Recently, we have discovered non-apoptotic functions of Dronc which are crucial to sustaining proliferation following necrotic ablation. To determine if hemocytes are recruited to necrotic injuries and contribute to signaling, as seen in the undead model, we used several independently created lines that express fluorescent proteins within hemocytes. We found that hemocytes are not recruited to multiple forms of necrotic ablation. Thus, regeneration following necrosis in the wing imaginal disc likely occurs without immune cell signaling.

284S **PERK prevents rhodopsin degradation and retinal degeneration by inhibiting IRE1-induced autophagy** Ning Zhao, Ning Li, Tao Wang National Institute of Biological Sciences, Beijing

Chronic endoplasmic reticulum (ER) stress is the underlying cause of many degenerative diseases, including autosomal dominant retinitis pigmentosa (adRP). In adRP, mutant rhodopsins accumulate and cause ER stress. This destabilizes wild-type rhodopsin and triggers photoreceptor-cell degeneration. To reveal the mechanisms by which these mutant rhodopsins exert their dominant-negative effects, we established an *in vivo* fluorescence reporter system to monitor mutant and wild-type rhodopsin in *Drosophila*. By performing a genome-wide genetic screen, we found that PERK signaling plays a key role in maintaining rhodopsin homeostasis by attenuating IRE1 activities. Degradation of wild-type rhodopsin is mediated by a selective form of autophagy, which is induced by uncontrolled IRE1/XBP1 signaling and insufficient proteasome activities. Moreover, upregulation of PERK signaling prevents autophagy and suppresses retinal degeneration in the adRP model. These findings establish a physiological role for autophagy in this neurodegenerative condition and indicate that promoting PERK activity could be used to treat ER stress-related neuropathies, including adRP.

285S **Stress granule nucleator Rin localizes to other oocyte RNP granules upon integrated stress response activation in oocytes** Rebecca Glineburg[1], Carolee Nguyen[2], Jackie Orellana Mejia[2], Hannah Adoian[2], Sophia Mansour[2], Jaclyn Pak[2] [1]Biology, Chapman University, [2]Chapman University

Ribonucleoprotein (RNP) granules have critical functions in both oogenesis and early embryonic development, and many respond to environmental stress. However, whether stress granules themselves exist as distinct RNP granules in *Drosophila* female germline cells and whether they impact oocyte cellular functions is unclear. Using an endogenously tagged fly line, we have identified the molecular requirements for stress granule formation in *Drosophila* oocytes and have characterized the cellular localization of the stress granule nucleator protein, Rin, in the presence and absence of stress. We show almost all cells in the ovary, with the exception of midstaged oocytes, are able to form heat shock, sodium arsenite, and hypoxia induced Rin granules, but are unable to form Rin granules in response to nutrient deprivation or TOR pathway inhibition. Under certain stress conditions, Rin recolocalizes and interacts with P-body proteins Tral and Cup, suggesting potential crosstalk between RNP granules.

286T **Unveiling the epigenetic landscape of the *Drosophila melanogaster* histone locus using DiMeLo-seq** Thomas E OHaren, Leila Rieder Biology, Emory University

Histone proteins are critical for organizing the genome, and precise regulation of their expression is essential during development. Histone levels are especially important in the early embryo when cells divide rapidly. The canonical histone genes cluster in the genome as tandem, repetitive units (collectively called the histone locus) to allow for quick and precise regulation. Out of the ~100 repetitive units, it's difficult to define which genes are actively transcribed, as the histone coding sequences are nearly identical in sequence. Similarly, it is difficult to describe the epigenetic landscape of the locus, as the repetitive nature of the histone genes excludes traditional short-read techniques, such as ChIP-seq. The newly developed DiMeLo-seq (Directed Methylation with Long-read sequencing) circumvents this issue by utilizing antibody-directed DNA methylation as opposed to immunoprecipitation in order to probe regions of protein-DNA interactions. This allows for the capture and sequencing of long-reads (~10-100kb) that contain sufficient, unique sequences to map to even highly repetitive regions of the genome like the histone locus. Using antibodies targeting transcriptional machinery and well-studied histone modifications involved in epigenetic control of gene expression, we reveal the chromatin landscape of the highly repetitive histone locus. Through DiMeLo-seq targeting H3K27ac, H3K4me3, and phosphorylated RNA polymerase II, we can determine which histone genes are transcriptionally active or silenced. Overall, DiMeLo-seq can reveal the epigenetic landscape of previously unmappable regions to better understand how these marks control gene expression of repetitive regions in the early embryo.

287T **Testing models of insertional bias for a centromere-enriched non-LTR retroelement** Tyler McDermott[1], Bianca Planeta[1], Bryce Chabot[1], Asna Amjad[1], Harshita Akella[2], Cecile Courret[3], Savannah Hoyt[1], Rachel O'Neill[1,4], Amanda Larracuente[3], Barbara Mellone[1,4] [1]Molecular and Cell Biology, University of Connecticut, [2]University of Connecticut, [3]Department of Biology, University of Rochester, [4]Institute for Systems Genomics

The centromere is an essential chromosomal locus that serves as the site of assembly for the kinetochore, which in turn attaches to spindle microtubules to mediate accurate chromosome segregation during cell division. Centromeres are composed of specialized chromatin containing the essential histone H3-variant CENP-A and highly repetitive DNA. Retroelements are a class of transposons capable of transposing in the genome via the reverse transcription and re-insertion of their RNA product. These selfish genetic elements have been found associated within centromere across taxa, but how these elements accumulate at centromeric chromatin is unknown. The retroelement *G2*/*Jockey-3* (henceforth *Jockey-3*) is the only repeat shared among all five centromeres of *Drosophila melanogaster*. *Jockey-3* is the most highly enriched repeat in CENP-A chromatin immunoprecipitations and, although copies of it are found throughout the genome, it is significantly enriched at centromeres. Recent *Jockey-3* insertions are disproportionately associated with CENP-A chromatin, suggesting the element has evolved to target centromeric chromatin for its selfish propagation. *Jockey-3* is also present at the centromeres of sister *Drosophila* species, despite the high divergence in their centromeric satellites. To determine if *Jockey-3* preferentially targets the centromere for reinsertion, we designed transgenic fly lines containing an engineered inducible copy of *Jockey-3* (e*Jockey-3*) whose presence can be tracked by SNP barcodes and epitope-tagged ORFs. Upon activation of e*Jockey-3*, we observe lethality and infertility following ubiquitous and germline expression, respectively. Immunofluorescence staining of induced tissues shows evidence of DNA damage and mitotic delay. DNA damage occurs at centromeres with relatively higher frequency compared to the rest of the genome, suggesting insertional bias. Ongoing co-localization studies will determine if either of *Jockey-3*'s two ORFs localize to the centromere. A digital droplet PCR (ddPCR) assay targeting the *eJockey-3* SNP barcode shows copy number variation in embryos following maternal germline activation as well as in larvae following ubiquitous expression. Furthermore, ONT long-read sequencing is currently underway to identify the location of any *de novo eJockey-3* insertions occur within the genome. Collectively, these findings will allow us to determine if and how this retroelement displays a preferential insertional bias for CENP-A chromatin.

288T **Maintenance of genome function in the absence of histone H3.3** Jeanne-Marie E McPherson, Daniel McKay, Robert Duronio Genetics, Biology, University of North Carolina at Chapel Hill

Histone proteins organize DNA into chromatin, which regulates all DNA-dependent processes. The genome encodes two types of histones: canonical histones that are expressed only during S-phase, and variant histones that are expressed throughout the cell cycle. Canonical histone H3.2 and variant histone H3.3 are some of the most highly conserved proteins among eukaryotes, suggesting that each histone type performs unique functions. Despite this conservation, we do not know whether variant H3.3 function is mediated by its unique protein sequence or by its cell-cycle independent expression. Canonical H3.2 and variant H3.3 differ by just four amino acids, three of which in the histone core domain mediate interaction with different histone chaperone complexes that deposit either H3.2 or H3.3 containing nucleosomes into the genome. The fourth residue at position 31 is an alanine in H3.2 and a serine in H3.3, the phosphorylation of which has been linked to gene regulation. To uncouple H3 protein identity from timing of expression, we used CRISPR to generate mutants that express H3.2 from the endogenous *H3.3* genes (*H3.3*$^{H3.2}$). We found that *H3.3*$^{H3.2}$ mutants have reduced lifespan and locomotion defects. Mutation of the H3.3-specific residue S31 (*H3.3*^{S31A}) had no impact on lifespan and mild locomotion defects, demonstrating that *H3.3*$^{H3.2}$ mutant phenotypes are driven primarily by the histone chaperone-interacting amino acids. To understand how these interactions impact genome function, we performed ATAC-seq and RNA-seq in 1-day and 10-day old *H3.3*$^{H3.2}$ and *H3.3*^{S31A} mutant brains. We found that overall patterns of chromatin accessibility and gene expression do not change in either mutant at either age. These findings suggest that cells have mechanisms to sense and respond to changes in the histone pool to maintain genome function. We hypothesized that histone chaperones play a central role in this mechanism. We are testing this hypothesis by examining the impact of reducing histone chaperone dose on viability and H3 protein levels in *H3.3*$^{H3.2}$ animals, and by investigating whether changing the H3 pool alters histone:chaperone interactions. Our work has revealed that cells have robust mechanisms to maintain genome function when the histone pool is altered. Furthermore, our work has implications for both our fundamental understanding of gene regulation and disease, as histones and histone chaperones are mutated in multiple cancers.

289T **Histone Mutation Results in Mitotic Recombination at Hotspots in Pericentric Heterochromatin** Priscila Santa Rosa[1], Bob Duronio[2,3,4,5], Jeff Sekelsky[2,3,4,5] [1]Genetics and Molecular Biology, University of North Carolina - Chapel Hill, [2]Department of Biology, University of North Carolina - Chapel Hill, [3]Lineberger Comprehensive Cancer Center, University of North Carolina - Chapel Hill, [4]Integrative Program for Biological and Genome Sciences, University of North Carolina - Chapel Hill, [5]Department of Genetics, University of North Carolina - Chapel Hill

Mitotic recombination (MR) can lead to loss of heterozygosity (LOH) or chromosomal rearrangements. LOH via MR is a hallmark of many types of cancer, but the biological mechanisms responsible for MR are not completely understood. MR often occurs in hotspots regions that are susceptible to double-strand breaks, and occurs in the context of chromatin that is regulated by post translational modifications (PTMs) of histone proteins. Histone PTMs play important roles in DNA replication, gene expression/repression, DNA repair, and chromatin compaction. However, it is unknown whether or how histone PTMs affect MR. To study the effects of histone PTMs on MR, we used a histone replacement platform in *Drosophila melanogaster* to substitute endogenous histone genes with modified genes producing histones that cannot undergo PTMs. In a genetic assay for MR in the male germline (which does no undergo either meiotic and mitotic recombination), we tested different histone mutants and discovered that flies heterozygous for H4K20R or H4K20A mutations have a significant increase in MR. By genome sequencing we found that the crossovers occurred in 3 specific regions within pericentric heterochromatin, revealing the existence of fragile sites. MR in region 1 occurred in a 12kb region within a ~2MB gene and contains an AT-rich repeat; region 2 contains AT-rich repeats and does not contain any known genes; region 3 contains several genes and does not have AT-rich repeats. Some of these features have been associate with common fragile sites in human cells, but they are not well understood. We will test whether gene transcription or DNA sequence contribute to fragility through CRISPR-Cas9 gene editing. We are also building a fluorescent assay which will allow us to analyze MR in different somatic tissues. This project provides insights into the role of H4K20 in preventing MR-LOH and preserving genome stability.

290T **Contribution of Phosphorylation to HP1a Function** James C Walts[1], Nicole C Riddle[2] [1]Biology, The University of Alabama at Birmingham, [2]The University of Birmingham at Birmingham

The Heterochromatin Protein 1 (HP1) family are non-histone chromosomal proteins that function in the formation of heterochromatin and in transcriptional regulation. HP1 proteins are found in plants, animals, and fungi. HP1a from *D. melanogaster* can act both as a repressor and an activator of transcription. Like many other proteins, HP1a undergoes post-translational modifications such as phosphorylation. However, little is known about the functions of HP1 post-translational modifications. To advance our understanding of HP1a's post-translational modifications, we produced two HP1a mutants that either mimic or block phosphorylation. Specifically, we replaced serines (S) 89/90/91 (S89/90/91) either with glutamic acid (E) to mimic permanent phosphorylation or with alanine (A) block phosphorylation. Using these mutant strains, we investigated how phosphorylation of HP1a impacts its known functions using two genetic backgrounds. Homozygous phospho-mimic animals were viable, while homozygous phospho-block animals did not survive past embryonic stages in both backgrounds. Both phospho-mimic and phospho-block HP1a mRNA is stable and accumulates at normal levels compared to wildtype HP1a. However, Western blot analysis demonstrated that while both phospho-mimic and phospho-block HP1a proteins are stable, they accumulate at levels lower than wildtype HP1a. Position effect variegation assays indicated that both phospho-mimic and phospho-block HP1a alleles act as suppressors of variegation, albeit to a lesser degree than an HP1a null allele. On the organismal level, we found that homozygous phospho-mimic HP1a mutants have a significant reduction in fertility and gonad size for both males and females compared to heterozygous and wildtype animals, but only in one of the two genetic backgrounds we examined. All males carrying one or more mutant HP1a allele and homozygous phospho-mimic females were more active when 24 days or younger compared to a wildtype control, a phenotype that is not seen in older animals. All mutant females also showed a lifespan extension, while only heterozygous phospho-mimic males showed an extension and heterozygous phospho-block males showed a decreased in lifespan compared to wildtype. Together, our results demonstrate that both phospho-block and phospho-mimic mutations impact the silencing function of HP1a, and that this effect might be due to a decrease in HP1a protein levels. However, this loss of silencing function does not necessarily lead to poor organismal health, as females with the mutations show an increase in lifespan. These results suggest that phosphorylation of HP1a proteins at the site we modified (S88/89/91) might impact HP1a protein stability and affect HP1a's silencing function. Furthermore, the results suggests that these sites might have specific functions in the Drosophila germline in genetic background dependent manner.

291T **Unique and redundant functions of canonical and variant histone residues and their modifying enzymes** Harmony Salzler, Vasudha Vandadi, Hina Sultana, Greg Matera Integrative Program for Biological and Genome Sciences, UNC Chapel Hill

Animal cells are thought to employ histone post-translational modifications (PTMs) to regulate fundamental developmental processes. Direct attribution of these functions to specific histone tail residues has been difficult due to inherent redundancies within histone multigene families, and their variants. Further complicating interpretation, most chromatin-modifying enzymes have non-histone substrates. To parse the relative contribution of histone tail residues and their cognate writer enzymes, we have developed a histone gene replacement system. Histone H3 lysine-36 trimethylation (H3K36me3), deposited co-transcriptionally by the lysine methyltransferase (KMT) Set2, marks gene bodies. Our previous work showed that gene expression changes due to H3K36 and Set2 mutation are context-dependent and often in apposition. The extent to which these properties are mediated by H3 variants (H3.2 vs H3.3), non-histone functions of Set2, or other H3K36 methyl states is unclear. Here, we employ genomic approaches in *H3.2K36R*, *H3.3K36R*, and *Set2Δ* mutants.

Transcriptomic profiling of *Set2Δ* and *H3.2K36R/H3.3K36R* (combined *K36R*) mutants reveals that most differentially expressed genes (DEGs) trend in opposite directions. Bioinformatic clustering of RNA- and ChIP-seq data suggest that Set2 is repressive in active contexts and activating at heterochromatic genes, whereas the situation is reversed in combined *K36R* animals. Cut&Run chromatin profiling of acetylated H4 shows that DEGs with the highest degree of upregulation in *Set2Δ* mutants are marked by the highest levels of gene-body hyperacetylation. We conclude that active vs repressive chromatin status is a key determinant of a Set2/H3K36 context-dependent regulatory switch. These and other findings not only suggest that K36 methyl-states have reciprocal functions, but also provide the first unequivocal evidence for the existence of Set2-dependent, K36-independent metazoan gene regulation mechanisms in *Drosophila*.

292T **Overlapping Role of m6A Dependent and Independent Genes in Cellular Processes and Alternative Splicing** George Boateng-Sarfo[1], Lijuan Kan[2], Sarah Signor[1,3], Eric Lai[2] [1]Genomics, Phenomics and Bioinformatics, North Dakota States University, [2]Developmental Biology program, Memorial Sloan Kettering Cancer Center, [3]Biological Sciences, North Dakota State University

m6A is the most prevalent internal RNA modification in eukaryotes, regulating critical cellular processes like cell development, differentiation, memory, and mRNA stability. It has been implicated in mRNA topology through exclusion from splice-adjacent regions by the exon junction complex. m6A is installed by a methyltransferase complex that includes METTL3 and its presence is interpreted by two YTH domain proteins. Although m6A has been implicated in many cellular processes, many studies are conflicting as to its exact role in splicing and gene expression. This is due in part to the lack of genetic resources employed in many studies such as knockout and gain of function samples, which restricts a comprehensive analysis of m6A-specific regulatory impacts. To bridge these gaps we generated maps of m6A modifications in several different cells types, including both gain of function and knockout samples. This is intended to be a sort of meta-analysis, to make broader conclusions about the tissue specificity of m6A while improving our understanding of its role in splicing and gene expression. Then we performed differential gene expression and splicing analysis. Our findings show that knockout of m6A and m6A readers (YTH domain proteins) promote exon inclusion, whereas gain of function of m6A readers in the m6A knockout background favors exon exclusion. These results shed light on the functional roles of m6A-specific splicing in RNA metabolism pathways and gene expression regulation.

293T **Genetic architecture of transposable element-mediated formation of heterochromatin in the euchromatic genome** Kayla Ly, Yuheng Huang, Anthony Long, Grace Lee Ecology and Evolutionary Biology, University of California, Irvine

Transposable elements (TEs) are mobile genetic elements that can replicate and "jump" within eukaryotic genomes, often compromising host fitness by disrupting crucial gene functions in gene-rich and transcriptionally active euchromatin. To counteract these harmful effects, host genomes typically silence euchromatic TEs through small RNA-mediated enrichment of repressive epigenetic marks. The resulting accumulation of repressive marks at silenced TEs can "spread" to adjacent sequences up to 20kb, potentially silencing neighboring regions—a phenomenon we term "TE epigenetic effects". These effects parallel the well-documented spreading of repressive epigenetic marks from transcriptionally inert constitutive heterochromatin. However, it is unclear whether genes known to modulate the spreading of repressive marks from constitutive heterochromatin can be directly extrapolated to euchromatic TEs, given that these TE-mediated enrichments of repressive marks are embedded within large stretches of active euchromatin. Here, we aim to identify genes that modulate the local enrichment of repressive marks at euchromatic TEs. Specifically, we use extreme-QTL (X-QTL) mapping to identify naturally occurring genetic variants contributing to TE epigenetic effects. X-QTL powerfully combines traditional QTL mapping with bulk segregant analysis, where individuals displaying extreme phenotypes are selected from synthetic multiparent populations and pool-sequenced to identify causal variants. Using globally collected wild-type founder strains from the Drosophila Synthetic Population Resource (DSPR), we identified natural genetic variation contributing to varying TE epigenetic effects. We quantified these effects using transgenic reporter strains in which the fluorescence intensity of a reporter gene proxies the extent of spreading of repressive marks from an adjacent TE insertion. To conduct X-QTL, we are crossing our reporter strains to an outbred population of the DSPR recombinant inbred lines (RILs) and comparing the genetic composition of F1 individuals exhibiting extreme fluorescence, and thus TE epigenetic effects, to identify genetic variants contributing to varying epigenetic regulation of TEs. Our study aims not only to identify previously unknown genetic factors modulating TE epigenetic silencing but also to deepen our understanding of how natural variation influences host regulation of euchromatic TEs.

294T **High-throughput transposition analysis of hybrid dysgenesis in *Drosophila virilis* and the impact of DNA damage on transposon dynamics** Ekta Mohanty, Justin Blumenstiel Ecology and Evolutionary Biology, University of Kansas

Transposable elements (TEs) act as genetic parasites, often causing DNA damage and potentially impacting genome stability. In *Drosophila*, hybrid dysgenesis (HD) results in sterility due to the activation of paternally inherited TEs in the germline. Females that lack these specific TE families cannot provide corresponding piRNAs, leading to uncontrolled TE mobilization and subsequent dysgenesis. To characterize the global transposition profile during HD in *D. virilis*, we are employing Oxford Nanopore sequencing. Our initial approach involved pooled sequencing of backcross progeny to identify novel TE insertions. However, to further refine the approach, we are developing a single-fly analysis method to track both TE insertions *and* excisions, allowing us to compare transposition rates in the germline of flies under dysgenic and non-dysgenic conditions. This revised approach will provide deeper insight into whether piRNA asymmetry globally predicts transposition rates and help explain variation in transposition activity across individuals. Furthermore, we aim to explore how transposons respond to DNA damage. By knocking down the DNA repair protein Spn-A (Rad51) through germline RNAi, we aim to assess its impact on transposon expression. This targeted approach will contribute to answering the broader question of how DNA damage influences transposon activity, potentially revealing whether DNA damage actually activates TEs by disrupting the function of the piRNA machinery.

295T **Spatial interactions between transposable elements and pericentromeric heterochromatin induce trans-allelic epigenetic effects** Yi Gao, Grace Lee Ecology and Evolutionary Biology, University of California, Irvine

Paramutation describes the trans-homolog interaction in that one allele epigenetically converts the state of the other allele. In flies, paramutation has primarily focused on a limited number of loci, needing a global picture of the prevalence and importance of this interchromosomal crosstalk of epigenetic states. In this study, we investigate the presence, prevalence, and functional impacts of paramutation-like epigenetic changes induced by transposable elements (TEs). TEs are "jumping genes" that can copy and move to other genomic locations at the expense of host fitness. TEs can have deleterious effects on the host's fitness. To suppress the selfish replication of TEs, hosts epigenetically silence TEs by depositing repressive histone modifications, such as H3K9me2/3. These repressive histone modifications not only "spread" into TE-neighboring genes and perturb gene expression but also mediate the 3D interactions between euchromatic TEs and pericentromeric heterochromatin (PCH). We predict that this interaction will influence the epigenetic states of adjacent sequences both in *cis* and in *trans* due to homologs pairing in *Drosophila* somatic cells. Accordingly, TE-PCH interactions on the TE-present allele could drag the homologous TE-absent allele into the suppressive PCH domain, leading to epigenetic changes of both homologs (trans-allelic enrichment of repressive histone modifications). To test this possibility, we crossed two wild-type inbred strains that have been fully sequenced with long-read sequencing and TE-annotated. We performed CUT&Tag on embryos to assay the epigenetic states around heterozygous TEs (presence/absence) and tested if the enrichment of repressive epigenetic marks, H3K9me3, is also present in the TE-absent allele and whether this depends on 3D interactions. Our preliminary analysis already found examples where the presence of TEs on one chromosome leads to the enrichment of H3K9me3 on the homologous sequence without TEs. We are now testing the effect of such trans-allelic enrichment of H3K9me3 influencing gene expression. Results from this study will provide a comprehensive understanding of TEs and their involvement in genome regulation by linking the harmful consequences of TEs and their role in shaping 3D genome organization.

296T **SET8 and H4K20me1 Control of DNA Replication During Early *Drosophila* Development** Karla I Troncoso[1], Aaron T Crain[2], Robert J Duronio[3] [1]Biology, University of North Carolina at Chapel Hill, [2]Life Edit Therapeutics, [3]Biology, The University of North Carolina at Chapel Hill

To duplicate large genomes during the short cell cycles characteristic of early animal development, cells initiate replication at thousands of origins throughout the genome. Although the machinery and regulatory mechanisms that govern DNA replication are conserved across eukaryotes, metazoan genomes are distinguished by the absence of DNA sequence specificity at origins of replication. Rather, origin specification and replication initiation is thought to be controlled by the local chromatin environment, which is largely modulated by post-translational modifications (PTMs) on histone N-terminal tails and the proteins that interact with these modifications. The 20th lysine in histone 4 (H4K20) can be mono-, di-, or trimethylated. H4K20 monomethylation (me1) has been implicated in DNA replication by analyzing mutations of the enzyme responsible for establishing this mark, SET8. A limitation to this approach is that SET8 has non-catalytic functions and non-histone substrates. Consequently, phenotypes observed from the perturbation of SET8 cannot be solely attributed to the loss of H4K20me1. To delineate the individual contributions of SET8 and H4K20me1 to DNA replication we engineered a single arginine-to-proline substitution, SET8^{RP}, within the catalytic domain of SET8 that is predicted to eliminate catalytic activity. *Drosophila* homozygous for this allele are viable, unlike a null allele which is lethal. *Set8^{RP}* females lay eggs, but these eggs never hatch regardless of the paternal genotype, a phenomenon indicative of a defect in early embryogenesis. The first phase of *Drosophila* embryogenesis is characterized by rapid and highly synchronous cycles of DNA replication and mitosis, without gap phases, that occur within a shared cytoplasm. Evaluation of embryos from *Set8^{RP}* mothers shows that despite there being appropriate amounts and proper localization of SET8^{RP}, nuclei in these embryos have defects including a high incidence of anaphase bridges, abnormal distribution, and variable nuclear size. Evaluation of DNA replication via EdU labeling shows variable amounts of EdU incorporation per nucleus within an individual embryo, indicating a defect in replication. Surprisingly, this essential role for SET8's catalytic activity appears to be independent of H4K20me1, suggesting that SET8 has substrates essential for DNA replication other than H4K20.

297T **Analysis of chromatin modifications at Dmef-2 enhancer during myogenesis** Sara Khadraoui, Sarah Anglin, Scott J Nowak Molecular and Cellular Biology, Kennesaw State University

The nuclear transcription cofactor Akirin plays a key role in the regulation of Dmef2 (*Drosophila* myocyte enhancer factor 2) during the early steps of embryonic myogenesis. Akirin is thought to help regulate *Dmef2* expression levels by mediating an association between chromatin remodeling complexes and Twist transcription factor activity. Previous work by the Nowak laboratory has determined that Akirin genetically and physically interacts with either the Brahma (SWI/SNF) chromatin remodeling complex, or through genetic interactions with the Nucleosome Remodeling and Deacetylase Complex. These interactions are essential for proper cardiac and skeletal patterning and development during embryogenesis. While both complexes have seemingly contradictory activities, the exact nature of covalent histone modifications that occur at *Dmef2* enhancers during myogenesis remains unknown. Using a variety of antibodies targeting various covalent histone modifications in chromatin immunoprecipitation we have begun an analysis of chromatin modifications that occur at the *Dmef2* enhancers during early, mid, and late embryonic myogenesis in both wild-type and *akirin* mutant backgrounds. Our results indicate that the histone modification landscape at the *Dmef2* enhancer is highly varied in *akirin* mutant backgrounds, which supports previous studies indicating that recruitment of chromatin remodeling complexes to these loci during myogenesis is key for their proper expression levels.

298T **Elucidating a bi-level neuronal function for Tip60 HAT and it's distinct domains at the chromatin and RNA level** Christina M. Thomas, Bijaya Manandhar, Felice Elefant Biology, Drexel University

The histone acetyltransferase (HAT) Tip60 is an essential epigenetic mediator of neuronal transcriptional regulation and is implicated in Alzheimer's disease (AD). Tip60 contains a catalytic HAT domain that promotes histone acetylation mediated chromatin control and a chromodomain (CD) that interacts with methylated histone lysine residues. Recently, our lab reported a novel RNA binding function for Tip60 that is localized within its CD and underlies neuronal RNA alternative splicing (AS) regulation in the brain. AS of RNA is a process that enables brain cells to generate different functional variants of the same protein to promote the protein diversity required for dynamic brain function in making new memories. Recent reports highlight defects in RNA splicing of genes in the brains of AD patients, thus making splicing disruptions a widespread hallmark of AD. Unfortunately, causes for these splicing disruptions in the brain are currently unknown. To further elucidate Tip60's RNA interaction function, we carried out high resolution homology modeling and molecular visualization of Tip60's chromodomain (CD), which strongly predicted a RNA binding loop within Tip60's CD critical for direct Tip60-RNA interaction. To tease apart Tip60's RNA versus histone binding function, we mutated highly conserved amino acids (a.a) in Tip60's CD strongly predicted to specifically interact with either histones ($Tip60^{mutHis}$) or RNA ($Tip60^{mutRNA}$) and generated transgenic flies carrying these inducible mutant Tip60 constructs. These transgenic fly models will serve as powerful tools to tease apart neural functions dependent upon histone vs. RNA binding or both. We will induce expression of mutant Tip60 in the fly brain and carry out functional assays to assess cognitive ability using both larval and adult learning and memory assays as well as assess brain morphology using immunohistochemistry. We will also assess gene expression using RNA-Seq, Tip60 splicing activity using rMATs on RNA-Seq data, and chromatin and RNA binding using ChIP and RIP, respectively. We anticipate that RNA versus histone binding functions are required for specific functional outputs and some neuronal processes will be more dependent on a given Tip60 binding function than others. Our results will elucidate a new bi-level regulatory role for Tip60 in chromatin and RNA that has potential to transform how researchers view Tip60 HAT mediated neural gene control in the context of cognition and AD.

299F **Meiotic nondisjunction in *Blm* mutant *Drosophila* and selective pressures in male progeny** Jayden T Youngren[1], Connor Alexander[2], Abigail Brown[2], Brayden Graves[2], Ava Hasenoehrl[2], Eric Stoffregen[2] [1]PLMSS, Lewis-Clark State College, [2]LCSC

Drosophila embryos lacking the maternally-provided Blm DNA helicase suffer severe DNA damage, leading to lethality in most. The surviving progeny exhibit an extreme sex-bias, with females (*XX*) comprising more than 70% of the population. This skewed sex ratio correlates with the lower repetitive DNA content in the *XX* female genotype compared to the *XY* male genotype, due to the highly repetitive *Y* chromosome. *Blm* mothers have increased meiotic nondisjunction (ndj), resulting in aneuploid sex chromosome genotypes, including *XO* males, with no second sex chromosome. We hypothesized that the reduced repetitive DNA content during Blm-deficient development would confer a longer lifespan on *XO* males compared to *XY* males. To test our hypothesis, we genotyped flies throughout a lifespan assay. In support of our hypothesis, we found that *XO* males do exhibit longer lifespans than *XY* males. Additionally, there was a single *XO* male in our control group (progeny from *Blm* males instead of *Blm* females) which also exhibited increased survival. This led us to question whether *Blm* males also show increased meiotic ndj. To investigate this, we developed a genetic cross scheme to score large numbers of progeny.

300F **Contribution of MBT-mediated interpretation of methyl-lysines to the establishment of de novo Polycomb landscapes** Sean Johnsen, Daniel J McKay Genetics, University of North Carolina at Chapel Hill

Polycomb Group (PcG)-mediated epigenetic memory is a critical gene regulatory system enabling maintenance of cell fate decisions throughout animal development. The regulatory logic of the Polycomb system can be bisected into two distinct phases. First is an initiation phase in early embryos, wherein target genes initially repressed by transcription factors and packaged in "naive" chromatin states are targeted by the PcG complexes for epigenetic silencing. Second is a maintenance phase wherein target genes already enriched for PcG-associated chromatin states stably maintain this enrichment across cell divisions. While a tremendous amount of research over the last one hundred years has unveiled portions of the complex logic underlying PcG-mediated epigenetic memory, technical limitations have made it difficult to study the initiation phase. Because of these technical limitations, most experiments have disrupted PcG proteins after the maintenance phase has begun, meaning the PcG regulatory system has entered a steady state. We hypothesize that there are mechanistic features of the initiation phase that differ from the maintenance phase. In this work, we seek to understand the degree to which the information encoded by methylated lysines in the tails of histones H3 and H4 direct the initiation of PcG-mediated epigenetic repression. Two members of PcG complexes have been shown to recognize methylated histone tail lysines through their conserved MBT domains: Sex combs on midleg (Scm) and Scm-like with four MBT domains (Sfmbt). Sfmbt dimerizes with Pho (homolog of human YY1), a sequence-specific DNA binding protein which has been shown to be important in PcG targeting, to make the PhoRC complex. Scm meanwhile has been shown to mediate interactions between PhoRC and the other two major PcG complexes: PRC1 and PRC2. Prior investigations in the role of Scm and Sfmbt have implicated MBT-mediated mono-methyl lysine recognition in the maintenance phase of PcG repression. However, their role in the initiation phase remains unclear. Through rapid protein knockdown systems and subsequent epigenomic sequencing, we measure the contribution of MBT-mediated interpretation of methyl-lysines to the establishment of de novo Polycomb landscapes.

301F **Delayed lagging strand synthesis drives asymmetric histone incorporation and promotes progenitor cell reprogramming in the *Drosophila* male germline** Brendon E M Davis[1], Jonathan Snedeker[1], Rajesh Ranjan[1,2], Matthew Wooten[1], Savannah Barton[1], Vikrant Mahajan[1], Xin Chen[1,2] [1]Biology, Johns Hopkins University, [2]Biology, Howard Hughes Medical Institute

In the *Drosophila* male germline lineage, stem cells display asymmetric histone inheritance while non-stem progenitor cells exhibit symmetric patterns. We report that an essential molecular mechanism underlying this cellular specificity is delayed lagging strand synthesis, which drives old histone incorporation into the leading strand and is sufficient to generate asymmetric histone incorporation in non-stem cells. A candidate screen identified that proteins involved in lagging-strand synthesis, such as DNA Polymerase α (Polα) and DNA Polymerase δ (Polδ), are expressed at reduced levels in stem cells compared to non-stem cells in the same germline lineage. Genetically compromising Polα induces the replication-coupled histone incorporation pattern in non-stem cells to be indistinguishable from that in stem cells, and this is recapitulated using a Polα inhibitor in a concentration-dependent manner. Furthermore, stem cell-derived chromatin fibers display a spatiotemporal difference in the replication of both DNA strands and show a significantly higher degree of old histone recycling to the leading strand than in progenitor cell-derived fibers. However, upon reducing Polα levels in non-stem cells, the progenitor cell chromatin fibers now display asymmetric old histone recycling just like stem cell-derived fibers. Importantly, these altered chromatin features in progenitor cells allow them to act like stem cells under both pathological and physiological conditions in which *bona fide* stem cells are lost. We have found no evidence of increased DNA damage in progenitor cells following the compromise of lagging strand synthesis, likely in part due to increased levels of Replication Protein A (RPA) in the germline. Together, these results indicate that developmentally programmed expression of key DNA replication components underlies asymmetric histone incorporation and could lead to differential cell fates following mitosis. Manipulating even a single DNA replication component can induce replication-coupled histone dynamics in non-stem cells that resemble the dynamics observed in stem cells.

302F **Knockout of Meiotic P26 in the Drosophila melanogaster brain improves passive avoidance behavior while its overexpression is lethal.** Steven Bradley, Elena S. Pak, Shallinie THANGADURAI, Alexander K. Murashov CBS, Louisiana State University

Meiotic P26 (Mei-p26), a protein widely studied for its role in germline cells, has also been shown to affect neural gene expression and behavior in adult Drosophila melanogaster. Our lab began investigating Mei-p26 after finding it increased in proteomic screening of offspring brains following a paternal obesogenic diet. Analysis with TargetScan and MIENTURNET, web tools for miRNA target prediction, revealed Mei-p26 as a potential target of miRNA-10, an evolutionarily conserved miRNA located in the Hox gene cluster. GAL4-UAS knockdown of miR-10 and miR-1006, a miRNA with an identical seed region, revealed fold changes in Mei-p26 that are inversely related to miR-10 and parallel to miR-1006, indicating that Mei-p26 is involved in miRNA regulation. Behavioral assays revealed hyperphagia in miR-10 knockdown flies and decreased consumption in flies without Mei-p26. This effect becomes especially pronounced when a dopaminergic *Ddc* driver line is used, indicating a potential link to reward-seeking regions of the brain. Mei-p26 knockout flies also outperformed controls in passive avoidance assays and had decreased nighttime activity, indicating better sleep and improved memory consolidation. We also observed lethality when we overexpressed Mei-p26 in the brain under an *Elav* promoter. While the role of Mei-p26 in the brain was previously unknown, recent studies suggest it may have an inhibitory effect on Elav, a transcription factor responsible for genes associated with synaptic plasticity and axonal growth. Taken together, we hypothesize that the observed changes in behavior and associative learning could result from changes in synaptogenesis during development. Understanding this miRNA/Mei-p26/Elav crosstalk may provide insight into the epigenetic mechanisms of behavioral phenotypes originating from a paternal obesogenic diet.

303F **Rethinking the basis of dosage compensation of the Drosophila X chromosome** James A. Birchler Division of Biological Sciences, Univ Missouri

A popular model to explain X chromosome dosage compensation involves the Male Specific Lethal (MSL) complex that binds the male X bringing along a histone acetyltransferase (MOF) that enriches H4Lys16Ac, a typical mark of gene activation, to double the expression of the single X. Mutations in members of the complex were claimed to reduce the expression of the male X by about half but all these studies, wittingly or unwittingly, normalize the X expression to the autosomes making it ambiguous whether the X is reduced or the autosomes increased. Indeed, examination of those data or experiments determining the absolute expression indicates that the autosomes are inversely increased in expression and the X is not reduced (in general). Trisomy for the X chromosome in flies (metafemales) also shows dosage compensation for most genes and the major effect on the autosomes is a reduced amount with a peak at the inverse level (~67%). These results reflect the phenomenon of the inverse dosage effect of transcription factors in aneuploidy studies, documented now in maize, Drosophila, Arabidopsis, yeast, mouse, and human. The claim that the male X expression is reduced by half in *msl* mutants without modulating expression across the whole genome is unlikely, given the dosage sensitivity of transcription factors. When the MSL complex is disrupted, there is a redistribution of H4Lys16Ac to be uniform across chromosomes (Cytology: Birchler lab, 1999; ChIP-seq: Akhtar lab, 2021). Such an increase of the histone modification on the autosomes is consistent with the increased expression. Ectopic expression of MSL2 in females organizes the MSL complex on the X's with increased H4Lys16Ac, but there is no increase in expression, while targeting MOF alone to transgenes will increase expression in females without the MSL complex, indicating some component overrides the effect of H4Lys16Ac. Mutations in nucleoporin Megator (Capelson lab) have overexpression of the male X suggesting that they, and potentially other genes, influence the override process. In the context of the results of gene expression in aneuploids across the phyla, it is hypothesized that males and metafemales capitalize upon an inverse dosage effect of transcription factors to bring about dosage compensation of the X chromosome with the MSL complex in males preventing overexpression of the X and indirectly muting an inverse effect on the autosomes by sequestering away MOF.

304F **Identifying chromatin regulators controlling stochastic gene expression in the *Drosophila* eye** Katalina N Li, Marina L Curchitser, Alison J Ordway, Robert J Johnston Biology, Johns Hopkins University

Development is generally consistent across individuals of the same species. However, some developmental processes occur stochastically, producing random patterns that are unique to each organism. These stochastic processes are necessary for proper development – however, their underlying genetic mechanisms are poorly understood. Here, we investigate the chromatin dynamics at a stochastically regulated locus expressed in the *Drosophila* eye. Within the fly eye, R7 photoreceptors express either the Rhodopsin 3 (Rh3) or Rhodopsin 4 (Rh4) protein. This R7 subtype cell fate decision is controlled by the transcription factor, Spineless (Ss). During larval eye disc development, chromatin state is dynamic at the *ss* locus. First, in undifferentiated cells, *ss* is not expressed and chromatin is compact. In precursor cells, an early-acting enhancer drives expression of *ss* and opens chromatin at the *ss* locus. *ss* transcription then ceases in differentiating cells and chromatin remains open in 67% cells and re-compacts in the other 33%. In the terminal R7 cells, a late-acting enhancer drives *ss* expression in the 67% of cells with open chromatin (Ss^{ON} R7s). The 33% of cells with compact chromatin (Ss^{OFF} R7s) do not express *ss*. Ultimately, each adult eye expresses Rh4 (Ss^{ON}) in 67% of R7 photoreceptors and 33% Rh3 (Ss^{OFF}), yet each eye has a random and unique mosaic of these cell types. Here, we aim to identify regulators of chromatin dynamics at the *ss* locus. To achieve this, we are conducting an RNAi knockdown screen of over 100 chromatin modifier proteins. We are selecting candidates from the screen which, when knocked down, result in significant phenotypic differences in their ratio of Ss^{ON} R7s in adult eyes. One major group of interest, the Trithorax group (TrxG), generally plays a role in maintaining transcriptional activation. Our screen thus far identified the TrxG protein Ash2 as a candidate regulator of the *ss* locus, as knocking down *ash2* expression results in a significantly decreased proportion of Ss^{ON} R7s. Once we identify candidates regulating Ss^{ON} ratio in adults, we will use RNA and DNA FISH to investigate how these candidates regulate *ss* transcription and chromatin compaction states throughout development. Together, results from this project will contribute to our understanding of chromatin dynamics regulating stochastic gene expression in the fly eye.

305F **Investigating pharmacologically synthesized Tip60 HAT-selective activators as a potential epigenetic therapy for Alzheimer's and Parkinson's diseases** Gu Gu Nge[1], Christina Thomas[1], Akanksha Bhatnagar[1], Chakrika Aluri[1], Bijaya Manandhar[1], Sandhya Kortagere[2], Felice Elefant[1] [1]Biology, Drexel University, [2]Microbiology and Immunology, Drexel University, College of Medicine

Histone acetyltransferases (HATs), particularly Tip60, play an important role in neurodegenerative disorders (NDs) by regulating neural gene expression critical for cognition. Our laboratory has previously demonstrated a significant reduction in Tip60 HAT protein and inappropriately elevated repressor HDAC2 protein in Alzheimer's disease (AD) hippocampal tissues and *Drosophila* AD models. This imbalance disrupts histone acetylation homeostasis, leading to impaired synaptic plasticity and cognitive decline. Notably, genetic upregulation of Tip60 in *Drosophila* AD models successfully mitigates these deficits. Current pharmacotherapeutic strategies primarily target HDAC inhibition to restore acetylation balance, but are often hindered by off-target hyperacetylation effects. Therefore, enhancing Tip60 HAT activity presents a promising alternative therapeutic avenue. To pursue this approach, we designed, synthesized, and tested a series of novel Tip60 HAT activator compounds in vitro, confirming their high binding affinity and specificity for the Tip60 HAT domain. To evaluate their therapeutic potential in multiple NDs, we assessed these compounds in *Drosophila* models of AD and Parkinson›s Disease (PD) by feeding these compounds to the flies and using functional assays to measure their ability to rescue ND-associated phenotypes that include deficits in locomotion, learning and memory and lifespan. Thus far, our results demonstrate that these compounds effectively rescue locomotor deficits in both AD and PD models. We are also conducting RNA-seq and western blotting to quantify changes in Tip60 mediated neuroplasticity gene expression and protein production. We hypothesize that administration of these Tip60 HAT-selective activators will restore Tip60/HDAC homeostasis, thereby reversing pathological symptoms in AD and PD models. These findings hold potential to establish a foundation for epigenetic-based cognitive enhancing therapies targeting Tip60 HAT in multiple NDs.

306F **Sex-dependent Dietary Impacts on the Epigenetic Silencing of Transposable Elements** Hannah Lee, Grace Lee Ecology and Evolutionary Biology, University of California Irvine

Transposable elements (TEs) are “selfish” genetic elements that can copy themselves and “jump” to other locations within the genome. To combat the selfish replication of TEs, eukaryotic hosts deposit repressive epigenetic marks to prevent their transcription and movement. Interestingly, past studies indicate that specific dietary conditions can have profound influence on the epigenetic regulation of the genome. This is because metabolites derived from one’s diet can act as cofactors, inhibitors, or substrates of histone-modifying enzymes, influencing the addition or removal of epigenetic modifications. Yet, our understanding of how diet may influence the epigenetic silencing of TEs has remained limited. To address this knowledge gap, I used transgenic reporter strains in which an mCherry fluorescent marker is placed adjacent to a TE insertion to measure the degree of TE epigenetic silencing. The repressive epigenetic marks enriched at the TEs can spread into the adjacent mCherry gene, impacting the Drosophila mCherry expression. By rearing flies under standard and experimental dietary conditions (turmeric, ketogenic, and low-calorie), it was found that a low-calorie diet increased TE epigenetic silencing in both male and females. Interestingly, while females exhibited weakened TE epigenetic silencing on both ketogenic and turmeric diets, males indicated the opposite effect. In this study, we aim to investigate whether the sex differences in TE epigenetic silencing on altered diets between males and females are due to the effect of sex or the Y chromosome, which is known to influence the chromatin environment. We leverage the fact that, in flies, sex is determined by the dosage between the X chromosomes and autosomes, and, accordingly, one could generate females with a Y chromosome (XXY) or males without a Y chromosome (XO). Hence, I am testing the sex difference in response to ketogenic and turmeric diets by crossing our mCherry reporter strains to attached X^Y males and will evaluate whether the dietary responses of XX^Y females and XO males follow that of their sex or the presence/absence of the Y chromosome. Thus, this study will reveal how dietary conditions may influence the epigenetic regulation of genetic parasites.

307F **Comparative Genomic Analysis of Contig 27 on the Muller F Element of *Drosophila willistoni*** Amrit Singh, James E. J. Bedard Biology, University of the Fraser Valley

Evidence-based comparative genomics was used to annotate genomic DNA sequence of contig 27, spanning 83,000 bp on the Muller F element of *Drosophila willistoni*. The project was completed with the help of the Genomics Education Partnership (GEP) undergraduate student research initiative. In Drosophila species the Muller F element is of interest due to having heterochromatic characteristics but maintains a gene density similar to euchromatic regions. The annotation of contig 27 included locating protein coding start sites, stop sites and determining the exact coordinates of all exons that comprised the open reading frame of each gene ortholog. The fully annotated genome of *Drosophila melanogaster* served as the reference. Bioinformatics tools used included the GEP UCSC Genome Browser Mirror, NCBI BLAST, Small Exon Finder, Gene Record Finder, and the Gene Model Checker. These tools were used in conjunction with biological evidence, such as RNA seq reads, to successfully construct high quality gene models for multiple isoforms of 3 putative gene orthologs present on contig27: PlexA, toy, ATPsynbeta. PlexA codes for a GTPase activating protein that uses semaphorins as ligands. PlexA-PA/PD demonstrated 94.3% protein ID (%ID), while PlexA-PA/PF showed 91.6%ID. toy serves a crucial function in regulating eye development. Three isoforms were noted: toy-PA (84.7%ID), toy-PC (85.6%ID), and toy-PD (74.1%ID). ATPsynbeta (97%ID) is a gene that codes for the beta-subunit of ATP synthase, thus serving a role in generating ATP. The construction of high quality evidence-based gene models will contribute to a better understanding of the characteristics and evolution of the Muller F element within Drosophila.

308F **Large-scale chromosome changes in single nuclei of developing *Drosophila* embryos** Akshada Shankar Ganesh[1,2], Taylor M Orban[1], Romir Raj[1,3], Peter I Fatzinger[1], Anna Johnson[1], Sean M Riccard[1,2], Akhmed Zhanaidarov[1], Mayu Inaba[3], Jelena Erceg[1,2,4] [1]Department of Molecular and Cell Biology, University of Connecticut, [2]Institute for Systems Genomics, University of Connecticut, [3]Department of Cell Biology, University of Connecticut Health Center, [4]Department of Genetics and Genome Sciences, University of Connecticut Health Center

Chromatin is intricately organized in 3D space to support gene function and regulation. Despite remarkable advances in our understanding of genome packaging and gene expression, it remains elusive how parental genomes are accommodated in each cell to achieve diverse cellular identities. Additionally, the spatial organization of chromosome territories (CTs) are highly variable across different cell populations, raising critical questions about its impact on genome regulation during development. To that end, we use an Oligopaints-based approach to investigate genome organization during the critical developmental process of zygotic genome activation, when the embryonic genome awakens. Using transformative single-nuclei imaging, we observe large-scale chromosome changes in the genome and extensive pairing of CTs between parental homologous chromosomes. Furthermore, we investigate how perturbation of transcriptional activity and pairing may impact whole-chromosome organization. Taken together, this study will enhance our understanding of parental genome folding and regulation, which may inform strategies for chromosome-based disorders.

309F **Histone H4 limits transcription of the histone locus in Drosophila** Kami Ahmad[1], Matt Wooten[1], Brittany Takushi[1], Velinda Vidaurre[2], Xin Chen[2], Steven Henikoff[1] [1]Fred Hutchinson Cancer Center, [2]Johns Hopkins University

The expression of core histone genes is coupled to DNA replication of the genome to support chromatin packaging. In Drosophila, core histone genes are repeated in one locus as a 100-copy array and forms the Histone Locus Body; these multiple copies support varying rates of cell proliferation in different developmental stages and various tissues of the animal. We show here that the Drosophila Histone Locus Body contains a mix of active and silenced units. In the male germline reporter histone repeat units are strongly silenced, and we used this setting to test the dependence of expression on chromatin factors and histones. We find that silenced histone genes are induced in response to demand for histones, and from a selected survey we identify that only the H4 histone is required for reporter silencing. Further, histone H4 protein localizes to the Histone Locus Body and is most enriched immediately after S phase of the cell cycle. This argues for a role of histone H4 in coupling the demand for histones for chromatin packaging to histone gene expression. Binding patterns of the NPAT regulatory factor and RNA Polymerase II in K562 cells suggests that this regulatory principle also operates in human cells.

310F **Deciphering the repressive pathway that controls precision of X chromosome dosage compensation** Kavana Gonur Biology, San Diego State University

Dosage compensation is a process where one sex of a two-sex organism equalizes its sex chromosome expression to match the other sex. Currently, the dosage compensation mechanism utilized by the Drosophila melanogaster male remains incompletely understood. It is known to be an epigenetic mechanism, in which the male-specific lethal (MSL) complex binds to regions spanning most of the male's sole X chromosome, resulting in their two-fold upregulation to match the female's two X chromosomes. However, it is still unknown how this precise two-fold level upregulation is achieved. Our previous research has identified a nuclear pore protein called Megator (Mtor, known as Tpr in mammals), comprising the nuclear pore complex basket structure, to be involved in setting this two-fold level of male X upregulation. There, depletion of Mtor resulted in aberrant hyperactivation of the X chromosome, specifically in the male. This research aims to expand on this discovery in three aspects. The first aim is to use an RT-qPCR screen to identify interacting partners of Mtor that similarly regulate this pathway. This is being done by measuring dramatic increases in transcribed mRNA levels of specific X chromosome gene targets in candidate gene RNAi knockdowns in salivary glands. Secondly, any putative "hits" from this screen will be investigated through RNA sequencing, with the goal of seeing a similar expression profile to Mtor knockdown tissues. Finally, this research will elaborate on Mtor's function on chromatin. This is to be accomplished through screening for histone modifications that may appear/disappear in Mtor RNAi knockdown progeny compared to wild-type progeny through the visualization of salivary gland polytene chromosomes. This in-progress project hopes to uncover additional co-functioning partners of Mtor in achieving this transcriptional precision, as well as provide further insight into how dosage compensation of the male X chromosome is regulated at the transcriptional and chromatin levels.

311S **Off-target piRNA production from D. simulans genes RYBP and PlexinB and their potential for paramutation** Paris Golder, Martina Dalíková, Justin Blumenstiel Ecology and Evolutionary Biology, University of Kansas

Paramutation is an epigenetic phenomenon whereby one allele permanently alters the expression of another allele in a heritable manner, independent of the underlying DNA sequence. Originally discovered in maize, paramutation has been primarily studied in plants, with limited research in animals. Interestingly, the piRNA pathway that suppresses transposable element (TE) activity in the germline is responsible for at least two documented instances of paramutation in Drosophila, one in D. melanogaster and the other in D. virilis. While the prevalence of piRNA mediated paramutation is unknown, a recent survey identified two genes in D. simulans that are candidates for paramutagenic activity in wild strains. These two genes, RYBP and PlexinB, are both silenced in the germline and produce high levels of piRNA from both strands, indicating conversion to a dual strand piRNA cluster. However, this pattern was identified in one strain, and not another. To investigate whether these function as paramutagenic alleles, we will evaluate F1 hybrids for trans silencing via qPCR. If trans silencing is observed, we will backcross female F1 progeny and evaluate their offspring for propagation of the silencing. Continued silencing in the absence of the original silent allele would confirm a system of paramutation. This work will provide insight into the prevalence of paramutation as a modifier of gene expression.

312S **Identification and functional testing of Sperm Nuclear Basic Proteins in the jewel wasp, *Nasonia vitripennis*** Patrick Zhang[1], Kassandra Soriano[2], Patrick M. Ferree[3] [1]Department of Natural Sciences, Pitzer and Scripps Colleges, [2]Pitzer College and Scripps College, [3]Department of Natural Sciences, Pitzer College and Scripps College

Sperm nuclear basic proteins (SNBPs) are small, highly basic proteins normally expressed specifically in the male germ line that package the DNA of sperm cells. SNBPs include three main types: protamines, protamine-like proteins, and sperm-specific histones. Protamines consist of as much as 60% arginine, while protamine-like proteins are 50% arginine or less, and they are thought to originate evolutionarily from histones. SNBPs play important roles in condensing the sperm's genome into a highly condensed state that facilitates sperm motility and protection from oxidative damage. Despite their importance, SNBPs have been difficult to study because they are known to evolve rapidly, making their identification challenging. A recent study used a biochemical approach to identify protamines and protamine-like proteins in a handful of different insects, including the jewel wasp *Nasonia vitripennis*. In this insect, three protamine-like candidates were identified. To complement this strategy, we performed in silico analyses to validate these findings and identify other potential candidates that may have been overlooked using biochemistry. Utilizing a testis-specific transcriptome, we filtered transcripts from ~15,000 genes by expression level, isoelectric point (>10), and gene ontology. We found 22 highly expressed genes whose encoded proteins are predicted to be highly basic and have DNA binding properties. Interestingly, two of the proteins found biochemically are present in our filtered gene subset. While these proteins do not possess the HMG domain, which is typical of SNBPs previously found in Drosophila species, they do contain other domain types that are predicted to interact with DNA. They also contain an enrichment of arginine doublets, which are commonly seen in SNBPs of other organisms. To functionally test these two genes, we have begun experiments to target their transcripts for degradation in males undergoing spermatogenesis by using RNA interference. Our preliminary results are showing a male sterility effect for one of these candidates. The male sterility phenotype(s) of these genes are being examined by microscopic analysis of chromatin state changes during spermatogenesis and post-fertilization. This study will facilitate subsequent ones aimed at understanding SNBP function and how selfish genetic elements alter male-specific chromatin processes during wasp gametogenesis.

313S **A selfish B chromosome in the jewel wasp *Nasonia vitripennis* produces a single protein that localizes to the seminal vesicle** Anabhra Singh[1], Patrick M Ferree[2] [1]Pitzer College and Scripps College, [2]Department of Natural Sciences, Pitzer College and Scripps College

Eukaryotic genomes harbor a range of different selfish genetic elements, including B chromosomes, which are non-essential for the organism. Because B chromosomes are not needed, they are prone to loss. To counter this tendency, B chromosomes can be transmitted at super-Mendelian frequencies to progeny, an effect that can directly or indirectly lead to fitness costs to the organism. A compelling question is what molecular characteristics allow B chromosomes to achieve high transmission. A key to answering this question is ascertaining the repertoire of genes expressed by B chromosomes and identifying which of them are important for transmission. Our group is addressing these goals by examining a B chromosome known as PSR (Paternal Sex Ratio) in the jewel wasp, *Nasonia vitripennis*. PSR is strictly transmitted to progeny paternally. The presence of PSR causes complete elimination of the sperm's essential chromosomes, but not itself, during the first embryonic mitotic division following fertilization. Due to the haplo-diploid reproduction of *N. vitripennis*, this genome elimination event leads to the conversion of diploid, female-destined embryos into haploid males that can transmit PSR. Previously we used different genomic platforms to identify 75 PSR genes that are expressed in the testis. Using systemic RNAi, we found that one of these genes, named *haploidizer*, is necessary for genome elimination. An important question is whether this gene, or any others, encodes proteins that are functional. We recently performed proteomics on three different tissue types – the young pupal reproductive tract, the adult reproductive tract, and the adult seminal vesicle – from the wild type and PSR+ males. From this work, we found that *haploidizer* does not produce any detectable peptides, arguing that this gene functions via non-coding RNA. Only a single gene (NV116418026) produces a protein of 242 amino acids that was detectable in the adult male reproductive tract and the seminal vesicle, the site of sperm storage. This finding suggests that the protein may be transferred via the sperm into the egg. We used RNAi to target the transcript in PSR males. Targeting this gene had no effect on genome elimination. However, our preliminary results suggest that the PSR chromosome tends to become lost when transmitted from F1 sons produced by RNAi-treated fathers. This finding suggests that the encoded protein may function in the transmission of this B chromosome.

314S **Exposure of Eggs and Larvae to Microbial Volatiles Alters Gene Expression in the Heads of Adult *Drosophila* Post Metamorphosis and Development in *Aedes* mosquitoes** Yuqi Ma[1], Jovanni Nunez[2], Anandasankar Ray[2] [1]Neuroscience, University of California, Riverside, [2]Molecular, Cell and Systems Biology, University of California, Riverside

Organisms are continuously exposed to volatile compounds such as microbial metabolites and odorants from our food and environment. Our lab previously demonstrated that prolonged exposure to some of these volatile compounds can alter gene expression in *Drosophila* and mice, with changes potentially driven by histone deacetylase inhibition (HDACi). HDACs remove histone acetylation marks, which promotes closed chromatin formation and transcriptional repression. By inhibiting these proteins, these odorants increase acetylation, and hence, alter gene expression. Using cell lines, we demonstrated a selective increase in H3K9ac, a marker of transcriptional elongation. *Drosophila* larvae, which burrow and feed on fermented fruit, encounter a higher concentration range of some of these volatile compounds in their environments. Chromatin signatures are often retained as a form of molecular memory in cells. However, whether exposure in the larval stage has any long-term effects in the adults after metamorphosis remain unknown. In this study, we investigate how early-life exposure to HDACi odorants impacts the fruit fly (*Drosophila melanogaster*). To understand the impact of such microbial volatiles on gene expression, we selected one such odorant, diacetyl, which is enriched in fermenting fruits. As expected, larvae exposed to a low concentration of this odorant showed changes in gene expression. Surprisingly, the larval exposure had a profound effect on gene expression in the adult stage as well, even though the animals were removed from odorant exposure prior to pupariation. The adult brains showed transcriptomic alterations in genes involved in actin cytoskeletal organization and neuronal development, suggesting this may influence neuronal signaling and behavior. To further understand the mechanisms driving these differentially expressed genes, we are assessing alterations in chromatin accessibility and acetylation patterns to test whether some epigenetic modifications established in larvae persist into adulthood. We extend the study to mosquito larvae, which develop in water with fermenting vegetation and microbes. Interestingly, mosquito eggs reared in very low concentrations of HDACi odorants showed reduced hatching rates and delayed development, suggesting that microbial volatiles can impact embryonic development across species. To identify genes driving these phenotypes, we are analyzing transcriptomic changes across developmental stages. Together, these findings suggest that exposure to specific HDACi microbial volatiles in early life stages can influence the epigenetic state, gene expression, and developmental trajectories in adult insects. These findings serve as a model for humans as well since volatiles like diacetyl are widely prevalent in various foods, beverages, and even in the skin microbiome.

315S **A Novel HP1a Partner Regulating Heterochromatin and Telomeric Transposons in the Drosophila Germline** Kun Wu, Richard Chang, Andrew Garcia, Maria Ninova Department of Biochemistry, University of California, Riverside

Heterochromatin is a basic component of eukaryotic chromosomes that occupies repeat-rich and gene-poor genomic regions around centromeres, telomeres, and some interspersed islands, characterized by a high density of nucleosomes and low transcriptional activity. Despite its critical roles in maintaining genome integrity and transcriptome fidelity, the mechanisms that govern the organization and function of heterochromatin are still not fully elucidated. Here, we uncover a previously unexplored protein interactor of the central heterochromatin effector Su(var)205/HP1a in the Drosophila female germline. We demonstrate that this protein engages through its large intrinsically disordered region with the hinge domain of HP1a and is recruited to mirror HP1a binding patterns, including occupancy of centromeres, telomeres, and piRNA clusters. Null mutants generated by CRISPR/Cas9 excision are viable, but display age-related decline in female fertility and telomeric transposon upregulation in the ovary. Further genetic and molecular characterization of this factor led to a model that it acts downstream of HP1a and contributes to the silencing of certain heterochromatin loci. Interestingly, we also found that the physiological levels of this protein are maintained through an autoregulatory mechanism, while its ectopic overexpression in nurse and somatic cells triggers aggregation and nuclear exclusion, underscoring a delicate balance in normal heterochromatin homeostasis. Together, these findings provide novel insights into the regulation and function of germline heterochromatin essential for preserving the genome integrity and fertility.

316S **Understanding the role of NASP in early embryogenesis in Drosophila** Mohit Das[1], Eli Coronado Chavez[1], Anusha D Bhatt[2], Amanda A Amodeo[2], Jared T Nordman[1] [1]Biological Sciences, Vanderbilt University, [2]Biological Sciences, Dartmouth College

To fuel the rapid nuclear divisions in the early embryo, a large pool of soluble histones is maternally deposited into the embryos. While a high concentration of histones can lead to histone-mediated toxicity in somatic cells, maternally-deposited histones are stabilized by chaperones that also seem capable of suppressing the toxic effects of excess histones. We have recently identified NASP as the histone chaperone responsible for stabilizing the soluble pools of H3-H4 in Drosophila embryos. Depletion of NASP compromises the levels of soluble H3-H4 in early embryos. Unlike the H2A-H2B chaperone Jabba, depleting NASP from the early embryo leads to slower embryonic development and stalling in the earlier embryonic cycles. Based on work in other systems, it is still unknown if NASP functions in the cytoplasm or nucleus to control H3-H4 stability and whether NASP controls H3-H4 dynamics. Using a combination of genetic and quantitative live imaging approaches, we have begun to dissect NASP function in the early embryo. We demonstrate that NASP functions in the cytoplasm to control H3-H4 stability. Live imaging of embryos laid by flies with NASP-Dendra, however, reveal that NASP is nucleoplasmic and rapidly imported into the nucleus. Using a combination of NASP mutants and a H3-Dendra reporter, we are attempting to define the nuclear function of NASP during embryogenesis.

317S ***Drosophila*** **Y chromosome variation impacts survival in Blm-deficient embryos** Connor K. Alexander, Jayden Youngren, Abigail Brown, Ava Hasenoerhl, Brayden Graves, Eric Stoffregen Physical Life, Movement, and Sports Sciences, Lewis-Clark State College

Blm DNA helicase plays a crucial role in maintaining genome stability during development. Blm females exhibit a significant maternal effect lethality, with most of their embryos failing to survive to the larval stage. Among the few survivors, females *(XX)* are overrepresented compared to males *(XY)*. This sex-bias correlates with repetitive DNA content, as the *XY* genotype contains more repetitive DNA content than *XX*. To test whether *Y* chromosome variation affects Blm-deficient embryonic survival, we obtained naturally derived lines from global *Drosophila* populations. We crossed their *Y* chromosomes into a common genetic background and crossed these males to *Blm* females. The resulting progeny showed variable female:male ratios. We leveraged the increased meiotic nondisjunction in *Blm* females to assess *Y* chromosome-specific lethality by comparing the ratio of sex chromosome aneuploid to euploid survivors (*XO:XY* males and *XXY:XX* females). *Y* chromosomes associated with increased female:male ratios also showed elevated *XO:XY* ratios among surviving males, suggesting the exacerbated sex-bias is caused by increased *Y*-associated lethality. Notably, no *XXY* females survived, suggesting a repetitive DNA content load that is too high in the absence of Blm. To investigate potential mechanisms for the *Y*-associated lethality, we used a position effect variegation (PEV) assay to assess relative heterochromatin content of the *Y* chromosomes, a proxy for chromosome size for the entirely heterochromatic *Y* chromosome. Surprisingly, we found no correlation between *Y* chromosome size and Blm-associated male lethality, suggesting that it may be specific types of DNA repeats that require Blm helicase during early development, rather than bulk repetitive content.

318S **Tip60 as a Key Regulator of Alcohol-Induced Epigenetic Changes in *Drosophila* Ventrolateral Neurons** Christian D. Del Valle-Colón[1], Miguel J. Álvarez-Cortés[1], Sebastián I. Morales-Cancio[1], Airined Montes-Mercado[1], María F. Acevedo-Kury[2], Angélica M. Crespo-Rodríguez[1], Diego A. Rodríguez-Plaza[1], Nicolás L. Fuenzalida-Uribe[3], Ted Chang[3], Andrew Seeds[3], José L. Agosto[1], Alfredo Ghezzi[1] [1]University of Puerto Rico, Río Piedras, [2]University of Puerto Rico, Cayey, [3]Institute of Neurobiology, Medical Sciences UPR, San Juan

Alcohol use disrupts critical neurological, physiological, and behavioral systems, leading to complex adaptations in the brain that contribute to alcohol tolerance, dependence, and withdrawal symptoms — collectively known as Alcohol Use Disorder (AUD). Recent evidence suggests that some of these adaptations can involve changes in neural circuits controlling circadian rhythms and sleep/wake cycles. While it is known that modulation of gene expression is central to alcohol-induced neuroadaptations, the specific epigenetic mechanisms involved in this regulation remain poorly understood. In this study, we investigate the role of the histone acetyltransferase Tip60 in alcohol-induced neuroadaptations using *Drosophila melanogaster* as a model organism. We focus on the ventrolateral neurons (LNv) that express pigment dispersing factor (PDF), a neuropeptide that regulates circadian and sleep behaviors and is implicated in alcohol tolerance development in flies. Given Tip60's role in histone acetylation, we hypothesize that its activity in LNv mediates transcriptional changes that underlie alcohol tolerance and sleep disturbances. To test this hypothesis, we utilized the UAS-GAL4 system to specifically knock down Tip60 in LNv neurons and examined resultant changes in alcohol-induced behaviors. Preliminary findings indicate that alcohol exposure affects the morphology of LNv neurons and the release of PDF neuropeptide. To further understand these transcriptional changes, we applied the Isolation of Nuclei Tagged in Specific Cell Types (INTACT) technique to isolate nuclear RNA from LNv neurons post-alcohol exposure. By tagging LNv nuclear membranes with GFP and performing immunoprecipitation with an anti-GFP antibody, we purified LNv nuclei for RNA sequencing analysis 24 hours after alcohol exposure. Differential expression analysis identified 253 genes with significant changes in expression between control and alcohol-exposed flies. Notably, these genes were highly enriched for mitochondrial translation processes, suggesting potential mechanisms underlying alcohol-induced alterations in neuronal energy dynamics. This study provides insights into the transcriptional landscape of alcohol neuroadaptation and underscores the role of Tip60-mediated epigenetic regulation in AUD. This study highlights the potential of *Drosophila* as a model to unravel the epigenetic mechanisms underlying alcohol-induced neural adaptations, providing a platform for future research on neuroplasticity processes and the molecular bases of alcohol tolerance.

319S **Quantitative analysis of the impact of local chromatin organization on transcriptional dynamics** Noel Buitrago[1], Bomyi Lim[2] [1]Chemical and Biomolecular Engineering, University of Pennsylvania, [2]University of Pennsylvania

Initiation of transcription requires promoters and their regulatory enhancers to interact in spatial proximity. However, in the eukaryotic genome, enhancers and their target promoters are often separated by large linear genomic distances containing numerous non-target genes, calling into question how these elements reliably find each other in the crowded nuclear environment. Insulator elements serve a prominent role in this long-range enhancer-promoter communication by organizing local chromatin into loop-like domains, facilitating interaction within the loop and silencing interaction with elements outside of it. Despite their importance, systematic analysis on the properties of insulators and their effect on transcription is lacking. This study employs single-cell live imaging techniques to elucidate the dynamics of insulator-mediated chromatin looping and its role in transcriptional regulation. Our system consists of a single enhancer that regulates two equidistant promoters in cis. We show that a single enhancer can co-activate two target promoters in cis, such that transcriptional kinetics from two equidistant reporter genes are coordinated. Insulators are then introduced in various positions and orientations to analyze their effect on transcription. Insulator-mediated looping of the system synchronizes gene activity and increases transcriptional output. Interestingly, bidirectional insulator conformations produce a more pronounced effect than unidirectional ones, indicating an orientation-dependent stability in insulator pairing. Insertion of an insulator between enhancer and promoter results in a sharp decrease in transcriptional output of the blocked reporter gene. Surprisingly, the blocked promoter is able to escape silencing and often exhibits coordinated transcriptional activity with the unblocked promoter. These results challenge the traditional view that insulator-mediated chromatin loops are largely static and suggest that the dynamic nature of insulator pairing contributes to transcriptional regulation to varying degrees, based upon their relative positions and orientations. Taken together, this work enhances our understanding of the relationship between local 3D chromatin architecture and transcription, providing new insights into the complex mechanisms governing gene expression.

320S **GCNA regulates the accumulation of DNA-protein crosslinks at satellite DNA repeats in the Drosophila germline** Anirban Dasgupta[1], Judith L Yanowitz[2], Michael Buszczak[1] [1]Molecular Biology, University of Texas Southwestern Medical Center, [2]Obstetrics, Gynecology, and Reproductive Sciences, Magee-Womens Research Institute, University of Pittsburgh School of Medicine

Several germ cell specific chromatin regulators, including Germ Cell Nuclear Acidic Peptidase (GCNA), play critical roles in the maintenance of genome stability to ensure production of healthy and viable gametes. *Gcna* is conserved within all sexually reproducing species and is responsible for clearing DNA protein crosslinks (DPCs), including those that form due to Topoisomerase II malfunction. Failure to resolve these bulky adducts interrupts several chromatin processes and causes reproductive failure and embryonic lethality. Loss of *Gcna* results in numerous mitotic defects during oogenesis and early embryogenesis, including formation of chromosomal bridges that contain satellite repeat sequences. The mechanism of DPC resolution on these satellite repeats remains poorly understood. To investigate the functional relationship between GCNA and the satellite repeats, we generated *Gcna* KO-ΔSAT mutant flies and performed immunofluorescence staining experiments. Our results show that several *Gcna* mutant phenotypes are substantially suppressed by loss of these satellite repeats. Consistent with this observation, the *Gcna* KO-ΔSAT mutant females exhibit dramatic increases in fertility as compared to *Gcna* KO females. We confirmed an increase of DPC formation in the *Gcna* KO ovaries using the RADAR assay in combination with quantitative TMT proteomic analysis. Surprisingly, this analysis revealed elevated levels of RNA helicases and piRNA pathway regulators in *Gcna* mutant DPCs alongside TOPII. The abundance of these protein-specific DPCs was reduced in the *Gcna KO-ΔSAT* mutants. Together, our data suggest that GCNA acts to prevent DPC formation at specific satellite repeats. Future experiments aim to determine the molecular basis of interactions of GCNA with TOPII and piRNA family proteins and validate their involvement in preventing chromosome bridges from forming at satellite repeats. These experiments will help us gain insights into the molecular function of GCNA and how its associated genetic network helps to protect germ cell chromosomes from damage.

321S **Tip60 as a Key Regulator of PDF Neuropeptide Release in Alcohol-Induced Neuroadaptations** Omaris Y De Pablo-Crespo[1], Christian D Del Valle-Colón[2], María de la Paz Fernández[3], Alfredo Ghezzi[2] [1]Biology, University of Puerto Rico - Río Piedras Campus, [2]University of Puerto Rico - Río Piedras Campus, [3]Indiana University Bloomington

Alcohol consumption is a leading cause of global mortality, contributing to approximately 2.6 million deaths annually, or 4.7% of all global deaths. It is linked to over 200 health conditions, including liver diseases, cancer, cardiovascular disorders, and injuries. Alcohol use disorder (AUD) is characterized by compulsive alcohol consumption, impaired control, and long-term brain changes affecting reward and inhibitory control systems, driven by neurochemical synapses. Alcohol consumption significantly impacts the sleep/wake cycles and alters neuronal function through epigenetic mechanisms that has not been explored yet. In this study, we aim to determine the role of the histone acetyltransferase Tip60 during alcohol-induced disorders using a Drosophila model. Tip60 plays a pivotal role in regulating chromatin accessibility and transcription of genes, critical for neuronal processes. Tip60 has been shown to regulate the axonal growth of small ventrolateral neurons (sLNvs), a subset of pacemaker cells essential for sleep-wake cycles. These neurons produce pigment-dispersing factor (PDF), a neuropeptide critical for synchronizing and stabilizing sleep-wake cycles regulation. Dysregulation of Tip60 activity can alter PDF expression and impair the morphology of sLNvs, resulting in disrupted sleep-wake cycles. Due to its role in the acetylation of histones, we hypothesize that Tip60 activity in LNv mediates alcohol-associated transcriptional adaptations that result in alcohol tolerance and sleep dysregulation. To test this hypothesis, we use the UAS-GAL4 system to manipulate Tip60 expression within LNv and analyze alcohol-induced effects on the release PDF neuropeptide. Preliminary experiments showed that alcohol exposure alters neuronal morphology and the release of the PDF neuropeptide in Tip60 knockdown LNvs using RNA interference (RNAi). Together, our results suggest that Tip60 is a key epigenetic modulator of alcohol responses in these neurons. Understanding the interaction between Tip60-mediated epigenetic regulation and alcohol responses offers valuable insights into how alcohol impacts neuronal function and behavior.

322S **Investigating the role of ORF1 in the transposition of the centromere-enriched retroelement *G2/Jockey-3*** Bianca Planeta[1], Tyler McDermott[1], Barbara Mellone[1,2] [1]Department of Molecular and Cell Biology, University of Connecticut, [2]Institute for Systems Genomics, University of Connecticut

Chromosome segregation is mediated by the centromere, a specialized chromosomal region that serves as an assembly site for kinetochore proteins and an anchor point for microtubules, enabling accurate chromosome separation. The centromere is epigenetically defined by the presence of CENP-A, a histone H3 variant that is found interspersed throughout the nucleosomes of the centromere. While the presence of CENP-A at the centromere is conserved, there is significant variation in the underlying DNA sequence composition across species. In *Drosophila melanogaster*, the centromere resides on islands enriched in retroelements, which are flanked by simple satellite repeats. Interestingly, a single genetic element, the retroelement known as *G2/Jockey-3* (or *Jockey-3*), is found at all the centromeres. Although *Jockey-3* is found at other parts of the genome, 61% of *Jockey-3* copies are found at the centromere. *Jockey-3's* consistent centromeric presence as well as enrichment at the centromere suggest that *Jockey-3* may have a transposition preference for these genomic regions.

Jockey-3 is composed of two open-reading frames (ORFs). ORF1 encodes a putative zinc-finger domain protein and likely contributes to nucleic-acid protein interactions. ORF2, contains a putative nuclease and reverse-transcriptase domains, the two critical domains for transposition of this type of retroelement. A tagged-ORF2 protein preferentially colocalizes with the centromere only in the presence of ORF1. This suggests that the ORF1 protein product is a likely candidate to play a role in *Jockey-3's* transposition bias for centromeres. To investigate this, we designed an inducible, tagged transgenic construct of ORF1, ORF1-v5.

Using immunofluorescence staining and imaging, I found that when expressed in larval brains under the neural driver elav-Gal4, 14.96% of ORF1-v5 foci colocalize with the centromere in interphase cells, compared to 0% in uninduced controls, and 1.11% in brains expressing a FLAG-tagged R2 retroelement, which is expected to preferentially target ribosomal DNA . These results are consistent with the hypothesis that ORF1 contributes to the transposition bias of *Jockey-3* for the centromere. Future work will elucidate the exact mechanism of *Jockey-3* centromeric targeting and, more broadly, help us better understand how other retroelements target genome subregions for transposition.

323S **Investigating the centromere drive model in *Drosophila melanogaster*** Ruiyi Sun MCB, University of Connecticut

The centromere drive hypothesis proposes that centromeres are selfish DNA elements exploiting transposition and unequal crossover to expand in size increasing their chances to be incorporated into the egg in female meiosis. Expanded centromeres are hypothesized to be associated with certain fitness costs, resulting in positive selection for suppressors of centromere drive. This project aims to develop a tractable model to empirically test the centromere drive hypothesis in *D. melanogaster*. I am devising a system to create an expanded centromere using site-specific recombination, creating a duplication expected to add 68.5kb of CENP-A associated DNA to cen3. A fluorescent maker on the expanded cen3 will help assess the transmission bias of expanded cen3. With plenty of genetic and cytological tools in *Drosophila*, we plan to then identify the fitness costs of the expanded centromere and screen for potential kinetochore mutations that suppress centromere drive.

324T **Rapid evolution and the horizontal transfer of transposons in *Drosophila*** Almoro Scarpa[1], Riccardo Pianezza[1], Hannah Gellert[2], Anna Haider[1], Bernard Kim[3], Eric Lai[4], Robert Kofler[5], Sarah Signor[6] [1]Vienna Graduate School of Population Genetics, [2]Stanford University, [3]Princeton University, [4]Memorial Sloan Kettering Cancer Center, [5]Populationsgenetik, Vetmeduni Vienna, [6]North Dakota State University

Horizontal transfer of genetic material in eukaryotes has rarely been documented in short evolutionary timescales. Previous work documented the invasion of D. melanogaster by seven transposons in the last two hundred years. Here, we found two retrotransposons, Shellder and Spoink, which invaded the genomes of multiple species of the melanogaster subgroup in the past 50 years. Through horizontal transfer, Spoink spread in D. melanogaster during the 1980s, while both Shellder and Spoink invaded D. simulans in the 1990s. Likely following hybridization, D. simulans infected the island endemic species D. mauritiana and D. sechellia, as well as D. teissieri with both TEs after 1995. In addition, three more transposons invaded D. melanogaster in the last forty years. This described cascade of TE invasions only became feasible after D. melanogaster and D. simulans extended their habitats into the Americas 200 years ago, likely aided by human activity. These invasions have profound effects on the evolution of drosophilid genomes, for example D. melanogaster has increased its genome size by more than 1.2 MB in the last 200 years. Our work reveals that these rapid cascades of TE invasions, likely initiated by human-mediated habitat expansions, have a profound impact on the genomic and phenotypic evolution of many geographically dispersed species.

325T **Shrew is a young Drosophila specific gene that was shaped by selection for rapid embryonic development into an accelerator of its ancient sibling Twisted gastrulation** Stuart Newfeld[1], MaryJane Shimmel[2], Sangbin Park[3], Robert Connacher[2], Kavita Arora[3], Michael O'Connor[2] [1]Arizona State Univ, [2]Univ Minnesota, [3]University California, Irvine

The acquisition of novel functions by new genes is well documented. What is rarely revealed is the selective pressure that pushed the new gene to adopt its new function. Here we address this gap and report that selective pressure for rapid embryonic development shaped the young Drosophila specific gene shrew into an accelerator of its ancient progenitor Twisted gastrulation (Tsg). The context for Shrew and Tsg function in Drosophila is dorsal-ventral (D/V) axis determination in the early embryo. Developmentally, a fly embryo must establish its D/V axis prior to gastrulation. D/V patterning in Drosophila is accomplished by rapid redistribution of the Decapentaplegic (Dpp) signaling protein from lateral regions to the dorsal midline. Redistribution creates a gradient of Dpp signal strength with a maximum dorsally that diminishes ventrally. The fate of cells along the D/V axis depends upon their local Dpp concentration. Among the classical D/V patterning genes only Shrew has yet to be assigned a function. Our molecular data indicated that Shrew is an N-terminally truncated version of Tsg that does not have an independent function in D/V patterning. Instead, Shrew's role is to enhance the function of its sibling Tsg. The next question was what type of enhancement? The short timescale for D/V patterning suggested the hypothesis that Shrew speeds up Tsg functions. The logic is that the immobility of eggs creates strong selective pressure for adaptations to minimize their exposure to predation, parasitism, and environmental stress. Minimizing the length of time as an egg could be an effective adaptation. With Tsg present essentially in all Bilaterian species, the hypothesis predicts that Shrew is present only in the fastest D/V patterning species. Our phylogenetics analysis revealed that this is true. Experimental tests of the hypothesis utilized conditions that slowed down Drosophila development, such that an accelerator of Tsg was unneeded. In three trials, a subset of shrew mutants survived each time. Thus, the most likely explanation remains that selection for rapid embryonic development drove Shrew to become a Tsg accelerator. To our knowledge, this is the first example where the acquisition of a novel function by a new developmental gene is tied to an identifiable selective pressure.

326T **A GEP F Element Project using four *Drosophila* species provides insights into mechanisms of genome size expansion** Timothy J Stanek[1], Wilson Leung[2], Christopher D Shaffer[2], Cindy J Arrigo[3], Sarah CR Elgin[2], Christopher E Ellison[1] [1]Dept of Genetics, Rutgers University, [2]Dept of Biology, Washington University in St. Louis, [3]Dept of Biology, New Jersey City University

Eukaryotic genomes are generally larger than implied by their gene content. What drives this expansion? The small (only 5.2 Mb) Muller F Element of *Drosophila melanogaster* is almost entirely heterochromatic, but the banded portion of the chromosome (1.3 Mb) contains ~80 protein-coding genes, with expression levels similar to those of genes in euchromatic domains. In several *Drosophila* species, the F Element is significantly larger than in *D. melanogaster*. To identify the major contributors to F Element expansion, and to assess its impact on gene characteristics, we performed a study of four such species. We constructed chromosome-level genome assemblies for *D. kikkawai*, *D. takahashii*, *D. ananassae*, and *D. bipectinata* using short- and long-read sequencing data followed by Hi-C scaffolding. These assemblies show that the regions spanning the F Element genes have undergone 2- to 16-fold expansions compared to *D. melanogaster*.

For the past four years, Genomics Education Partnership (GEP) students have engaged in structural gene annotations of these four species in Course-based Undergraduate Research Experiences (CUREs). Analyses of these annotated assemblies show that transposable elements (TEs) and other repeats drive expansion of the F Element, primarily in intronic and intergenic regions. Natural selection appears less efficient on these expanded F Elements: they have smaller effective population sizes and their genes exhibit reduced usage of optimal codons, compared to *D. melanogaster*. Surprisingly, *D. ananassae* and *D. bipectinata* are the most closely related species in our study (8.7 Mya), yet their F Elements display a high rate of structural change, sequence evolution, and largely independent transposon-driven expansions. We propose that variations in F Element sizes are driven by differences in recombination rates. Although recombination on the *D. melanogaster* F Element has never been observed directly, population genetic analyses suggest that recombination does occur at low levels on this chromosome in the wild. On the *D. ananassae* and *D. bipectinata* F Elements, recombination is likely either absent or rare enough to allow TEs and other deleterious mutations to accumulate via Muller's ratchet, leaving these chromosomes more similar to a Y chromosome than to the F Elements of other *Drosophila* species.

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327T **Doublesex directs the differentiation of a new photoreceptor in the male housefly dorsal eye** Antoine Donati[1], Yunchong Zhao[2], Eleanor Terner[2], Michael Perry[2] [1]University of California, San Diego, [2]UCSD

Many insect species have evolved the ability to detect and chase rapidly moving preys or mates, and such behavior rely on a specialized region of the eye. One example is the "Lovespot" of the dorsal-anterior retina of the male houseflies (Musca *domestica*), which allows them to chase females at high speed in full flight. In Drosophila *melanogaster*, each facet of the eye contains 8 photoreceptors, with receptors 1 to 6 being more involved in motion vision and receptors 7 (R7) and 8 in color vision. Previous work has shown that in Musca *domestica's* Lovespot, photoreceptors 7 display a change in light sensitivity and axon projection that make them more suitable to detect motion along with photoreceptors 1 to 6. Studying the development of the Lovespot thus can help us understand how new cell types evolve in the peripheral nervous system to fit the organism needs.

Single cell RNA sequencing revealed a strong upregulation of the transcription factor Doublesex in Lovespot photoreceptor 7 (lsR7) along with an upregulation of the transcription factors spineless and defective proventriculus (dve) and a downregulation of the transcription factor spalt. CRISPR-Cas9 knock out of the R7-specific transcription factor spineless revealed that the strong upregulation of Doublesex in lsR7s depends on a positive feedback loop between Spineless and Doublesex. Knocking out doublesex showed that it is necessary for spineless and dve upregulation and spalt downregulation as well as for rhodopsin 1 expression and rhodopsin 2 and 3 repression in lsR7s.

Using pupal retina bulk ATACseq and piggybac transgenesis, we identify a R7-specific enhancer and use it to overexpress Dsx in all R7s at mid-pupal stages, showing that Doublesex can downregulate spalt and upregulate dve in R7s across the retina in both males and females. Using a combination of rhodopsin promoters, we show that driving sustained Doublesex overexpression from midpupation to adulthood in a subset of R7s distributed across the entire retina is sufficient to switch rhodopsin expression from Rhodopsin 2 to Rhodopsin 1. We are now trying to determine if this overexpression is also sufficient to change R7 axon projection.

328T **Morphological innovation without gene co-option: the *Drosophila* sex comb evolved via heterochronic and quantitative changes in general cellular processes** Ben R. Hopkins, Olga Barmina, Artyom Kopp University of California, Davis

How the evolutionary process innovates remains a central question in biology. One potential route is through the individualization of serially repeated homologs. Under this model, homologs are individualized by changes in the regulatory apparatus ('character identity network', ChIN) that activates the downstream gene networks that build them. Individualization decouples homologs from one another, providing regulatory separation that allows downstream gene networks to be rewired to reach new phenotypic endpoints. Despite this model's intuitive appeal, we have few examples that connect ChINs to changes in downstream gene expression. The *Drosophila* sex comb presents an especially promising model in which to test these ideas. Previous work has shown that a subset of mechanosensory bristles on the male *Drosophila* foreleg was individualized by the evolution of a novel ChIN, which consists of an autoregulatory feedback loop between the Hox gene *Scr* and the effector of sexual differentiation *dsx*. Here, we show that the new ChIN partly drives the transformation of a mechanosensory bristle into a sex comb tooth by accelerating shaft growth. Using time-series single-cell RNA-seq, we further show that this acceleration is driven by quantitative and heterochronic changes in gene expression in sex comb cells, rather than the co-option of new genes. This contrasts with a more ancient bristle homolog, chemosensory bristles, which appear to have co-opted multiple, organ-specific genes. We show that at different developmental stages, sex comb cells boost the activity of gene modules that regulate general cellular processes—such as ATP production, metabolism, and translation—and that this boost is facilitated by male-specific changes in endoreplication dynamics that result in higher ploidy in comb cells. Collectively, our work shows that morphological innovation can proceed without the co-option of new genes into downstream trait-building networks, offering a simple, flexible route through which new traits can evolve.

329T **Recapitulating the horizontal transfer of a novel innate immune factor in *Drosophila*** Rebecca Tarnopol[1], Josephine A Tamsil[2], Gyöngyi Cinege[3], Ji Heon Ha[4], Kirsten I Verster[5,6], Edit Ábrahám[7,8], Lilla B Magyar[3], Bernard Y Kim[6], Susan L Bernstein[2,5], Zoltán Lipinszki[7,8], István Andó[3], Noah K Whiteman[2,5] [1]Plant & Microbial Biology, UC Berkeley, [2]Molecular & Cell Biology, UC Berkeley, [3]Innate Immunity Group, Institute of Genetics, HUN-REN Biological Research Centre, [4]Data Science, UC Berkeley, [5]Integrative Biology, UC Berkeley, [6]Biology, Stanford University, [7]Synthetic and Systems Biology Unit, Institute of Biochemistry, HUN-REN Biological Research Centre, [8]National Laboratory for Biotechnology, Institute of Genetics, HUN-REN Biological Research Centre

Immune systems are among the most dynamically evolving traits across the tree of life. Long-lived macroparasites have played an outsized role in shaping innate immunity in animals. Insects are excellent models for illuminating the strategies that animals evolved to neutralize such enemies, including nematodes and parasitoid wasps. One such strategy relies on endosymbioses between insects and bacteria that express phage-encoded toxins as well as horizontal transfer of the genes that encode the toxins to insects. Here, we used genome editing in *Drosophila melanogaster* to recapitulate the evolution of two of these toxin genes — *cytolethal distending toxin B* (*cdtB*) and *apoptosis inducing protein of 56kDa* (*aip56*) — that were horizontally transferred likely from phages of endosymbiotic bacteria to insects millions of years ago. We found that a *cdtB::aip56* fusion gene (*fusionB*), which is conserved in *D. ananassae* subgroup species, dramatically promoted fly survival and suppressed parasitoid wasp development when heterologously expressed in *D. melanogaster* immune tissues. We found that FusionB was a functional nuclease and was secreted into the host hemolymph where it targeted the parasitoid embryo's serosal tissue. Although the killing mechanism remains unknown, when expressed ubiquitously, *fusionB* resulted in delayed development of late stage fly larvae and eventually killed pupating flies. These results point to the salience of regulatory constraint in mitigating autoimmunity during the domestication process following horizontal transfer. Our findings demonstrate how horizontal gene transfer can instantly provide new, potent innate immune modules in animals.

330T **Transfer RNA gene repertoire expansion in *Drosophila*** Dylan Sosa, Jianhai Chen, Shengqian Xia, Marek Sobczyk, Tao Pan, Manyuan Long Ecology & Evolution, University of Chicago

Eukaryotic genomes encode hundreds of transfer RNA genes, ostensibly to ensure efficient translation. How these seemingly redundant copies arise and are naturally selected in genomes and populations remains largely unexplored. In this work we infer the tempo of *D. melanogaster* tRNA gene origination, their mode of evolution, and show that *Drosophila* tRNA isodecoding and isoaccepting genes have been continuously lost and gained throughout 60 million years of divergence leading to taxonomically restricted copies. We identify biased expression and fragmentation of lineage-specific tRNAs between tissues and indeed even between isotype copies that originated at different points during Drosophilid evolution. We experimentally assess phenotypic effects of knocking out recently duplicated tRNA copies in *D. melanogaster* and their contribution to Darwinian fitness through assays of fertility and viability. Our findings provide evidence that the evolutionary process of eukaryotic tRNA gene repertoires is characterized by continuous changes in membership and is not influenced by the emergence of new protein coding genes.

331T **Convergent Evolution of Neo-Sex Chromosomes in *Zaprionus* Species** Ching-Ho Chang[1], Medhavi Verma[2], Kevin Wei[3], Ryan Bracewell[2] [1]Fred Hutchinson cancer research center, [2]Department of Biology, Indiana University Bloomington, [3]University of British columbia

In many species, sex chromosomes have independently evolved from autosomes by acquiring sex-determining roles, leading to characteristic differentiation: X chromosomes retain most genes, while Y chromosomes lose gene content. Recent studies of neo-sex chromosomes—formed through fusions between autosomes and sex chromosomes—have provided critical insights into the evolution of sex chromosomes, shaped by mutation, drift, and selection. However, the relative impact of these forces is still debated, partly due to differences in sex chromosome origins across species. Here, we report two independent fusion events between autosomes and sex chromosomes in *Zaprionus* species (within the *Drosophila* subgenus), dating back approximately 15 million years. Both fusions involve Muller's element B, presenting a unique opportunity to trace the evolutionary trajectories of the same genes following fusion. Our results reveal increased expression of genes on both neo-X chromosomes in males, suggesting the acquisition of dosage compensation. Additionally, we observe amplifications of several genes on Y chromosomes in two different lineages. These findings suggest that sex chromosome evolution may be partially predictable based on gene function, highlighting convergent evolutionary patterns shaped by gene-specific function. This system also enables further exploration of the contributions of evolutionary forces, including Muller's Ratchet, Hill-Robertson interference, meiotic drive, and sexual conflicts, in sex chromosome evolution.

332T **Adaptive variants underlying melanism in high altitude Drosophila melanogaster are polymorphic in both ancestral and derived populations** Tiago da Silva Ribeiro[1,2], John E Pool[2] [1]University of São Paulo, [2]Laboratory of Genetics, University of Wisconsin - Madison

Understanding the genetic basis of adaptation is a central question in evolutionary biology. Empirical studies have shown distinct adaptive genetic architectures among species, traits, and populations. Here, we perform extensive quantitative trait locus (QTL) mapping experiments focused on the darkest known population of D. melanogaster, from high altitude Ethiopia, to investigate the genetic architectures underlying this instance of melanic evolution. We mapped three distinct pigmentation traits in 21 mapping crosses between dark strains from Ethiopia and light strains from a Zambian population from the species' ancestral range. QTLs overlapping the canonical pigmentation genes ebony, tan, and yellow were each present in just under half of all mapping experiments, and tended to have stronger phenotypic effects. Some additional QTLs overlapped with documented pigmentation genes, while other QTLs point to presently unknown contributors. We also performed mapping for a subset of crosses at a cooler, more Ethiopian-like temperature, which indicated thermally plastic effects on a minority of the QTLs that may have enhanced or resisted the evolution of melanism in Ethiopia. On average, we found that the Ethiopian and the Zambian parental strains involved in a cross were equally powerful determinants of the QTLs detected. These results are congruent with selection on relatively common pigmentation variants that were already present in the ancestral range, and rose moderately in frequency under local adaptation in Ethiopia but did not approach fixation. Thus, even for fly pigmentation traits often thought to have relatively simple molecular underpinnings, we find evidence that an abundance of standing genetic variation gave rise to persistently variable genetic architectures underlying adaptive traits in the evolved population.

333T **Characterizing Genetic Variation in Morphological Scaling** austin wilcox[1], Alexander Shingleton[1], Tony Frankino[2] [1]Biological Sciences, University of Illinois at Chicago, [2]University of Houston

Morphological scaling relationships between body and trait size capture the characteristic shape of a species, and the evolution of these scaling relationships is the primary mechanism that generates morphological diversity. Nevertheless, we have almost no understanding of the genetic architecture of morphological scaling, critical if we are to understand how scaling evolves. Here, we begin to explore the genetic architecture of population-level morphological scaling relationships – the scaling relationship fit to multiple genetically-distinct individuals in a population – by describing the distribution of individual scaling relationships – genotype-specific scaling relationships that are typically unseen. These individual relationships contain genetic variation influencing relative trait growth within individuals, and theoretical studies suggest their distribution shapes population-level scaling response to selection. Previously, we demonstrated extensive genetic variation in wing-body and leg-body nutritional scaling among 197 isogenic lines of *Drosophila melanogaster* – that is, individual scaling relationships generated by variation in developmental nutrition. Since insulin signaling regulates trait-specific nutritional scaling differences, we hypothesized that variation in insulin-signaling genes contributes to genetic diversity in individual scaling relationships. To test this, we conducted a genome-wide association study (GWAS) to identify loci associated with scaling variation across lineages and used functional genetics to validate genes linked to these loci. As hypothesized, these genes include members of the insulin-signaling pathway and the ecdysone-signaling pathway, which also regulates body and trait size in response to nutrition.

334T **Impacts of epigenetic silencing of transposable elements on local mutation rates** Yuheng Huang[1], Grace Yuh Chwen Lee[2] [1]UC-Irvine, [2]University of California, Irvine

Mutation is the ultimate source of genetic variation. Understanding the causes of varying mutation rates within and between species remains a central question of evolutionary genetics. Both sequence context and chromatin states can influence the rates and spectra of mutations. Intriguingly, some of these factors have been predicted to have conflicting impacts on mutations. For example, repressive epigenetic marks enriched in heterochromatin are thought to be associated with reduced occurrence of double-stranded breaks, which should lead to lower mutation rates. However, the same marks could also reduce the accessibility of DNA repair enzymes and, thus, error-prone repair, resulting in opposite impacts on mutation rates. Notably, in the euchromatic regions of the genome, repressive epigenetic marks are also found enriched at transposable elements (TEs), widespread genomic parasites, as a consequence of the host-directed mechanism to epigenetically silence TEs. To investigate whether this TE-associated epigenetic silencing in the euchromatic genome affects local mutation rates, we conducted mutation accumulation (MA) experiments using lines with varying levels of TE-mediated enrichment of repressive marks. Specifically, we transgenically altered the expression level of Su(var)3-9, a dosage-dependent modifier of position effect variegation (PEV) and known to show quantitative influence on the spreading of repressive marks from pericentromeric heterochromatin. Approximately 50 MA lines of transgenic strains with varying levels of Su(var)3-9 expression underwent full-sib mating for 22 generations. Using whole-genome short-read sequencing, we will compare the mutation rates for homologous sequences with and without repressive chromatin marks across transgenic genotypes. Besides point mutations, we will also study how the repressive chromatin marks impact indels and TE replications. Overall, this work will likely reveal whether TEs influence local mutation rates and whether TE-related chromatin modifications mediate this effect.

335T **Investigating how evolutionary changes in the *nanos* 3' UTR influences its function in translation and localization** Gisselle A Hidalgo[1], Ahad L Shabazz-Henry[1], Melissa M Menzel[1], Sunna Joseph[1], Kristina Spencer[1], Elizabeth R Gavis[2], Matthew G Niepielko[3] [1]School of Integrative Science and Technology, Kean University, [2]Molecular Biology, Princeton University, [3]School of Integrative Science and Technology, Kean University

The development and maintenance of the germline, the set of highly specialized cells responsible for passing on genetic material to the following generation, is essential for animal reproduction. Germline function and maintenance require the formation of highly conserved biomolecular condensates called germ granules. Germ granules contain many types of mRNAs and proteins that have important roles in germline differentiation, proliferation, and post-transcriptional gene regulation. Previously, we discovered a remarkable amount of natural diversity in the number of transcripts that accumulate within germ granules among species, which also reflected diversity in the number of coalesced primordial germ cells within their embryonic gonads, supporting a link between germ granule evolution and changes in germline development. In *Drosophila*, the accumulation of *nanos* (*nos*) within germ granules and its translational regulation requires the 3' UTR, which displays significant diversity among species. Interestingly, replacing *D. melanogaster*'s *nos* 3' UTR with other *Drosophila* species results in the increased presence of defective primordial germ cells, demonstrating a decrease in the robustness of germ granule function. Given the 3' UTR's dual function in the regulation of germ granule accumulation and translation, we tested whether the increased presence of defective primordial germ cells was caused by evolutionary changes in the 3' UTR that impacted accumulation efficacy and/or translational regulation. We found that different species 3' UTR's reduced *nos*'s ability to accumulate within germ granules while translational regulation remained highly functional. Together, our findings show that translational regulation by the *nos* 3› UTR is more conserved than its role in localization, supporting a model where evolutionary changes that affect mRNA accumulation within biomolecular condensates, rather than translation control, can influence condensate function.

336T **Evolutionary reversal of dominance in color dimorphism in the *Drosophila montium* species group is explained by evolution of phenotypic plasticity** Yuichi Fukutomi[1], Emily K Delaney[1], Alexandra Phillips-Garcia[1], Jingqi Liu[2], Ashley Chuang[3], Masayoshi Watada[4], Seema Ramniwas[5], Artyom Kopp[1] [1]UC Davis, [2]University of Virginia, [3]Evergreen Valley High School, [4]Tokyo Metropolitan University, [5]Chandigarh University

Allelic dominance is a fundamental factor determining the genotype-phenotype relationship. By changing the phenotype of heterozygotes, evolutionary reversal of dominance can contribute to adaptation or resolves sexual conflicts. Theoretical models predict that changes in gene expression can provoke dominance reversal, but experimental evidence is currently lacking. Abdominal pigmentation in the *Drosophila montium* species group is a suitable model to study the molecular mechanisms of evolutionary dominance reversal. Many species display a Mendelian color dimorphism with alternative Dark and Light phenotypes. GWAS analyses reveal that independent regulatory mutations in the POU domain motif 3 (*pdm3*) gene, a repressor of pigmentation, are responsible for the color dimorphism in multiple species. However, antibody staining and *in situ* hybridization do not show an obvious difference in the expression pattern of Pdm3 protein or mRNA between morphs. These results suggest that quantitative differences in gene expression levels may be responsible for phenotypic variation. Interestingly, the dominance relationship between the Dark and Light *pdm3* alleles differs between species. While the Dark alleles are dominant in most species, exceptions are found in *D. bocqueti* and *D. jambulina*. In those two species, the Light allele is dominant at high temperatures. In *D. bocqueti*, the Dark allele is dominant at low temperatures and the two alleles are co-dominant at intermediate temperatures. In *D. jambulina*, the dominance relationship is unstable at low temperatures; Dark, Light, and intermediate phenotypes are evenly observed in heterozygotes. In contrast, no thermal plasticity is observed in the other species. The results suggest that changes in the dominance relationship between alleles can be explained by the evolution of phenotypic plasticity. We propose a hypothesis that abdominal pigmentation in the *montium* clade is a threshold trait, where continuous variation in gene expression is converted into discrete phenotypes. Under this model, quantitative differences in gene regulation between species underlie the evolution of both phenotypic plasticity and allelic dominance.

337T **Species-specific Acetobacter microbiota influence on Drosophila fitness and host adaptation** JiaSyuan Chen[1], Shu Fang[2], Chau-Ti Ting[1] [1]Department of Life Science, National Taiwan University, [2]Biodiversity Research Center, Academia Sinica

Animals interact continuously with microorganisms throughout their lifespan, driving host-microbiota coevolution and potentially fostering species-specific commensal relationships. While some microbial species form stable, long-term associations with their hosts, most host-microbe interactions are transient. The degree to which these interactions are flexible, however, remains poorly understood. In this study, we investigated the microbiota composition across multiple *Drosophila* species, including *D. melanogaster*, *D. simulans*, *D. sechellia*, and *D. yakuba*, and identified Acetobacter as the predominant bacterial genus, with notable species-specific variation in composition. We isolated several key Acetobacter species—*A. pasteurianus, A. persici,* and *A. thailandicus*—from these *Drosophila* species and created mono-associations with germ-free flies to assess the effects on the fitness of the host species. The results showed that different Acetobacter species had varying impacts on embryo development time and offspring number. These findings highlight the complex interspecies interactions between flies and bacteria, emphasizing the roles of microbiota in host adaptation. Our results suggest that microbial symbionts contribute to heritable phenotypic diversity, potentially aiding animal hosts in environmental adaptation.

338T **Evolutionary dynamics of the nuclear export factor gene family across *Drosophila* genus** Jae Hak Son, Maria Volski, Christopher Ellison Rutgers University

Nuclear export factors (Nxf) are proteins that have a conserved and critical role in transporting RNA from the nucleus to the cytoplasm. In *Drosophila melanogaster*, Nxf1 (also known as Sbr) mediates the export of most of the mRNA and the export of piRNA precursors originating from uni-strand piRNA clusters. Nxf2 lost its RNA export ability and instead form a protein complex with Panx and Nxt1 that interacts with Piwi for co-transcriptional silencing of transposons. Nxf3 is involved in exporting piRNA precursors originating from dual-strand piRNA clusters in the germline. Nxf4 shows testis-specific expression but its specific function remains unknown. *Drosophila* Nxf family can have diversified their molecular functions, not only for the RNA export but also for the host genome defense against transposons. We identified members of the Nxf family using 106 *Drosophila* species to examine the dynamic evolution of the Nxf family across the *Drosophila* genus. A phylogenetic analysis of all Nxf peptides showed that Nxf2 – 4 are experiencing accelerated evolution across the genus, compared to Nxf1, which is responsible for the ancestral mRNA export function of the gene family. We confirmed that these four gene family members were present in the common ancestor of all *Drosophila*species, however, each family member has also experienced multiple duplication events as well as losses in various *Drosophila* lineages, with *nxf4* showing the most duplications and the most losses. We also observed a tendency for the duplication of one family member to be accompanied by the loss of another member, raising the possibility of functional compensation among *nxf* paralogs. To test for the presence of functional complementation, we measured the sex bias of expression level for each *nxf* gene family member in over 19 species of *Drosophila*. These results showed a tendency for the loss of nxf4 to be accompanied by the duplication of another *nxf* family member with the duplicate copy displaying the testis-biased expression. Together, our results show that this gene family is evolving rapidly and dynamically, potentially related to its role in TE silencing. Furthermore, the testis-specific function of Nxf4 may have evolved repeatedly across multiple lineages as losses of the parental *nxf4* gene were offset by testis-specific duplications of other gene family members.

339T **Expression level of tandem duplications of the *Adh* gene is not always 2-fold, it depends on which sequences are duplicated** David Loehlin Biology, Williams College

Tandem duplication of genes is an important mutation process in disease, individual variation, and evolution. A basic hypothesis is that duplication of a whole gene would result in twice the expression level, but deviations from 2-fold from tandem gene duplications are often observed. A key question is whether deviations are influenced by the size of the duplicated segment, the content of the segment, or both. To investigate these factors, we engineered 25 different tandem duplications of the *D. melanogaster* gene *Alcohol dehydrogenase* (*Adh*) and compared their expression. The duplications were produced from combinations of 5 distinct left-hand and 5 right-hand junctions using “recombinase-mediated tandem duplication” (Loehlin et al. 2023) and range in duplicated block size from 4.2 to 25 kb. Expression level of many of these otherwise-identical *Adh* duplications deviates from twice the single-copy level. We will present the pattern of expression variation and the relative contributions of duplicated segment size and sequence content.

340T **Phenotypic and genetic basis of reproductive isolation in recently diverged fruit fly populations** Myron B Child[1], Matthew Lollar[1], Eleyna Escobedo[2], John Pool[1] [1]Genetics, UW Madison, [2]UW Madison

Deciphering how diverging populations become reproductively isolated is crucial to understanding how new species are formed. Many studies that focus on long divergent, distinct species, however, miss the early stages of this process and are unable to explore where the first sources of isolation originate. Using inbred strains of Drosophila melanogaster generated from recently isolated European and African populations, we find evidence of hybrid breakdown, wherein F2 males generated from between population crosses show elevated levels of sterility. This increased sterility is variable, however, dependent on the cross direction and wild strain used. Dissections show that the phenotypes of sterility are variable even within a single cross, and include testes morphological defects, spermatogenic defects, and ineffective sperm transfer. This suggests a variable and complex multigenic basis for reproductive incompatibility between populations that have only recently separated. Continuing efforts to map pairwise incompatibilities between these inbred strains will shed light on the genetic basis for the observed sterility and whether chromosomes from both African and European populations are implicated. Overall, our results support the idea that the initial incompatibilities that arise between allopatric populations are due to multiple accumulated Bateson-Dobzhansky-Muller incompatibilities.

341T **Artificial selection in wild-derived *Drosophila* experimental populations and effects on stress and behavioral resistance** Elizabeth Everman University of Oklahoma

Heavy metal pollution has pervasive environmental, health, and evolutionary impacts. In humans, health risks range from permanent neurological disease to increased morbidity of degenerative syndromes. My previous work has demonstrated that variation in susceptibility to metal toxicity is influenced by a combination of genetic variation, environmental exposure, and their interaction. My lab focuses on copper given the important role this metal plays as an environmental contaminant and as a critical micronutrient for normal physiological development and maintenance in most organisms. Through my work with natural and laboratory *Drosophila* populations, it has become clear that copper resistance has a complex genetic basis and is influenced by the physiological status of the individual as well as the ability to perceive and behaviorally respond to stressors in the environment. I collected flies from two potentially copper contaminated environments and am currently evolving copper resistance in replicate experimental populations using an evolve and resequence approach to assess copper, lead, cadmium, and starvation resistance. By measuring these traits throughout selection, I am tracking and characterizing the genetic control of evolved responses to copper stress. I am examining the effects of adult selection on developmental resistance to understand the ontological consequences of selection, and I am examining variation in copper avoidance throughout selection to identify possible behavioral contributions to evolved resistance. Using an integrative approach that leverages whole genome and RNA sequencing and experimental evolution, we will track the dynamic shifts in allele frequency and gene expression in response to copper stress in diverse naturally derived genetic backgrounds. By focusing on multiple physiological and behavioral traits, these approaches will provide critical insight into the interconnectedness of multiple response traits, while also illuminating genetic factors that influence behavioral and learning disabilities linked to metal poisoning.

342T **Adaptive piRNA pathway tuning tames sex- and lineage-specific selfish genes** Peiwei Chen[1], Alexei Aravin[2] [1]Cornell University, [2]California Institute of Technology

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

343T **Mitonuclear genetics of performance traits under OXPHOS complex 1 inhibition reveals pervasive epistasis and genotype-by-environment interactions** Leah M Darwin[1], Yevgeniy M Raynes[2], Faye A Lemieux[2], Jacob D Lerman[2], Jack H. Blocker[2], Olivia M Maule[2], camille M Brown[2], David M Rand[3] [1]Computational Biology & Ecology, Evolution and Organismal Biology, Brown University, [2]Department of Ecology, Evolutionary and Organismal Biology, Brown University, [3]Ecology, Evolution and Organismal Biology, Brown University

Understanding the roles of epistasis and genotype-by-environment interactions in the architecture of complex traits is a central challenge of population, evolutionary and quantitative genetics. We have developed a mitonuclear genetic approach to address these questions through experimental pairings of mtDNAs and nuclear genotypes. Proper cellular function requires coordinated expression of the 37 mitochondrial-encoded and >1200 nuclear-encoded genes that have been interacting for more than 1 billon years. Disruption of these complex interactions can cause a variety of diseases and influence adaptive evolution in heterogeneous environments. To advance our understanding of how these complex genetic interactions influence organismal fitness we have constructed a panel of 88 mitonuclear genotypes built from 22 different mtDNAs (10 Zimbabwe, 10 Bejing, from D. melanogaster plus D. simulans and D. yakuba mtDNAs) placed onto two nuclear chromosomal backgrounds (OreR and DGRP375) in duplicate. Each genotype was cultured on standard Drosophila diet and a diet containing 25 mM rotenone, a natural pesticide that inhibits the activity of mitochondrial OXPHOS complex I, NADH dehydrogenase. Climbing speed, flight ability, development time and body weight were measured in adult males and females from each diet, totaling >125,000 flies. There were strong main effects of mtDNA, nuclear genotype and diet on each trait but mitonuclear epistatic effects and GxE effects differed across traits. Climbing speed and development time showed clear mitonuclear epistatic and mito-diet GxE effects. The outgroup mtDNAs of D. simulans and D. yakuba had climbing and flight performances comparable to D. melanogaster mtDNAs despite ~100-150 amino acid altering substitutions in the protein coding genes of these mtDNA genomes. There was no correlation between mtDNA divergence and phenotypic divergence as mtDNA effects were largely explained by epistasis and GxE. Overall, the analyses identify specific mtDNA and nuclear genome pairs that show sensitivity or resistance to rotenone, amenable to further genetic mapping of epistatic and environmental interactions.

344T **Evolution of piRNA clusters in the *Drosophila simulans* ovary.** Prakash Narayanan[1], Dr.Sarah Signor[2] [1]North Dakota State University, [2]Biological Sciences, North Dakota State University

Transposable elements (TEs) are mobile DNA sequences that selfishly increase in copy number in the genome. The transposition of TEs is largely deleterious, and to prevent this the host has a dedicated system for suppressing their movement that relies primarily on small RNA termed piRNA. piRNA is bound by Argonaute class proteins that recognize transcripts cognate to the small RNA and initiate silencing transcriptionally and post-transcriptionally. piRNAs originate from discrete loci in the genome called piRNA clusters (piC). Little is known about the evolution of piCs. The two popularly proposed models of piRNA cluster evolution are the *'trap'* model and the *'de novo'* model. The '*de novo'* model posits that the piC formation is driven by individual TE insertions and is not sensitive to genomic location. The ‹*trap' models suggest* that a TE invading a novel host genome continues to transpose until a copy lands in a piC. Once the copy is inserted into a piC, piRNA cognate to the TE is produced and transposition is suppressed. We used five inbred genotypes of D. simulans to call genotype-specific piC and test several hypotheses that will distinguish between these two models of cluster evolution. The results of the study suggest an extensive variability in the size and frequency of piRNA clusters between strains, but consistency within a strain across generations. Larger clusters were shared by more strains, but piC private to a single genotype was much more numerous. This suggests that the de novo model applies to cluster birth, and while many low-frequency clusters are lost piC expands in size as they increase in frequency in the population. Indels in piC are also longer than those outside of piC, reflective of the fact that full-length TEs are inserted into piC. We also saw a difference in SV type distribution when comparing unique clusters to clusters shared by all strains. Unique clusters had more insertions, while shared clusters exhibited a balance between SV types. Our study suggests that piC evolution may not be divided into two models but instead can be a continuum that includes characteristics from both. The results support the idea that piCs are primarily born '*de novo*' and a few select clusters from this formation turn into stable and larger clusters by trapping more TE insertions.

345F **A Category Theory-Based Framework for Modeling Host-Transposable Element Dynamics** Shashank Pritam, Sarah Signor Biological Sciences, North Dakota State University

We present a novel computational framework for modeling the evolutionary dynamics of *insect endogenous retroviruses* (iERVs) using *category theory and functional programming* principles. Our approach leverages monadic composition and functorial mappings to capture the complex interplay between host genomes and transposable elements, implementing population genetics forces through pure functions and algebraic data types. The framework represent state transitions as morphisms in the ‹category of types›, with functors capturing the relationship between genotype and phenotype spaces, while emphasizing referential transparency and immutable state management. Through validation against empirical datasets from Drosophila melanogaster, we demonstrate that our model predicts iERV distribution patterns and reveals that host-TE dynamics follow distinct phase transitions, particularly in the relationship between infective and non-infective iERV populations. Based on our extensive simulations across multiple host generations, we predict this framework will identify previously unknown stability points in host-TE fitness trajectories, showing that successful transposable element invasion occurs predictably when host fitness costs remain low, with invasion rates declining sharply as fitness costs increase beyond critical thresholds.

346F **A potential gene model for intralocus sexual conflict resolution by translocation to the Y chromosome in *Drosophila*.** Eduardo G Dupim[1], Gabriela Matias[1], Rafael Vaz[2], Antonio Bernardo Carvalho[3], Rodrigo Cogni[2], Maria D Vibranovski[1] [1]Department of Genetics and Evolutionary Biology, University of São Paulo, [2]Department of Ecology, University of São Paulo, [3]Department of Genetics, Federal University of Rio de Janeiro

Gene translocation from autosomes to the Y chromosome has been widely cited as a possible solution to intralocus sexual conflict (ISC). Although supported by theoretical models and genomic evidence there are few experimental studies on this topic reinforcing the need for suitable study models. Here, we investigate the origins and evolution of the Y-linked gene *kl-2*, a male fertility factor encoding a dynein protein of ~4450 aa predominantly expressed in testes, associated with sperm flagellar motility in *Drosophila* fruit flies. *kl-2* was originally located on an autosome and was translocated to the Y chromosome leaving a copy in its autosomal ancestral position: *CG9068*. This gene suffered severe size reduction, encoding a truncated protein with ~1200 aa that retained only the first functional domain. Comparative genomics of dipteran species revealed that the duplication event occurred in the common ancestor of the Drosophilinae subfamily, with both copies conserved in all analyzed species, ruling out the hypothesis of *CG9068*'s pseudogenization. Moreover, we found that *kl-2* has undergone independent duplication to the Y chromosome in the *Phortica* genus, another branch of drosophilids (Steganinae subfamily), where the autosomal copy experienced size reduction similar to *CG9068* in *Drosophila*, suggesting convergent evolution process. RNA-Seq analysis shows that *CG9068* is specifically expressed in the second antennal segment in *Drosophila melanogaster*. This segment contains auditory sensory cells with ciliary motility, indicating that the truncated gene might play a similar role to *kl-2* in spermatozoa. Comparative analysis of the mosquito *Aedes aegypti* and the drosophilid *Rhinoleucophenga americana* (subfamily Steganinae) revealed that the autosomal nonduplicated gene is expressed in both antennae and testes. Furthermore, the antennae exhibit alternative splicing, producing a protein with only the first domain similar to *Drosophila*'s *CG9068*. These findings strongly suggest that the ancestral gene was expressed in both hearing cells and sperm with each copy specializing in one cell type after its duplication. A potential ISC could exist between constitutive and male-specific isoforms with the latter being detrimental to females which was potentially resolved by the duplicative translocation to the Y chromosome followed by subfunctionalization. Functional experiments are being performed to address this hypothesis.

347F **"Ultra" long-read sequencing is required for faithful structural variant calling in D. melanogaster** James Hemker[1], Bernard Kim[2], Dmitri Petrov[3] [1]Developmental Biology, Stanford University, [2]Princeton University, [3]Biology, Stanford University

Structural variants are genomic rearrangements (>50bp) such as insertions, deletions, and inversions. Structural variants can be large-effect mutations with significant phenotypic impact, and they have been implicated in human disease. They have also been shown to be key adaptive mutations across the evolutionary tree of life. Due to their size and the often-complex genomic regions these variants are found in, long-read sequencing data is best suited to discover structural variants. Automated variant callers allow structural variants to be found rapidly at the genomic scale. However, long-read sequencing produces variable read lengths, and the effect of read-length on the accuracy of automated structural variant calling has not been extensively studied. Here, we used Nanopore long-reads to deeply sequence eight inbred D. melanogaster lines. D. melanogaster was an ideal system for this study due to their small, gene-dense genomes, and prior knowledge regarding large-scale variation. We computationally downsampled our total set of reads to smaller pools of variable read-length distributions. We ran each of these downsampled pools through the same structural variant calling pipeline, and then we manually validated the calls by looking at the raw read alignments of more than 13,000 genomic loci to ascertain the true positive rates for each read-length distribution. We found that variants found from “ultralong” reads (read N50 ~72 kb) had true positive rates of ~97% for variants both larger and smaller than 10kb in length. Variants less than 10kb that were called from “standard” long-reads (read N50 ~15kb) were similarly accurate, however large variants longer than 10kb had a true positive rate of just 78%. Additionally, we showed that short-read data called only 16% of variants longer than 10kb correctly. Finally, we conclude that having extremely long reads, as opposed to removing the shortest reads, has the greatest effect on improving structural-variant-calling accuracy.

348F **Quantifying The Selection Pressure on Structural Variants of Common Fruit Flies** Jen-Yu Wang, James J. Emerson Department of Ecology and Evolutionary Biology, University of California Irvine

Long-read sequencing technologies have significantly facilitated genome assembly and the discovery of structural variants (SVs). In this study, we assembled over sixty genomes of *Drosophila melanogaster* using long-read data from the NCBI SRA. We identified SVs, including deletions, insertions, duplications, inversions, and translocations, through an assembly-based pipeline validated by simulation. While mapping-based programs are computationally efficient, they exhibited lower sensitivity compared to assembly-based methods, which effectively captured larger-scale genomic changes.

Our findings indicated that insertions were generally larger than duplications, followed by deletions. Single nucleotide polymorphisms (SNPs) were found to be thousands of times more abundant than SVs. We explored the association between SVs and various types of repeat units. Insertions were primarily linked to retrotransposons, whereas deletions and duplications were more frequently associated with simple repeats and satellites. A detail often overlooked when only inspecting repeat elements in an assembly. Notably, around 30% of SVs contained more than one type of transposable element

Unlike SNPs, SVs, which often span and affect multiple genes, are more likely to influence an organism’s phenotype. This suggests stronger selective pressure on SVs, although the extent was previously unknown. In this study, we generated unfolded site frequency spectra for each category of SVs and transposable element superfamilies. Our results show that SVs are subject to much stronger negative selection compared to non-synonymous SNPs, by several orders of magnitude. We observed that selection signals varied among different types of SVs and transposable elements. Additionally, we investigated genes with specific functions or high allele frequencies to identify SVs that may contribute to population differentiation.

349F **A Lengthy Affair: The Genetic Basis of Seminal Receptacle Length and its Role in Reproductive Isolation** Amisha Agarwala, Yasir H. Ahmed-Braimah Center for Reproductive Evolution, Department of Biology, Syracuse University

Seminal receptacle (SR) length and sperm length exhibit patterns of rapid diversification and correlated evolution across various taxa, including the genus *Drosophila*. These patterns are generally attributed to post-copulatory sexual selection, and the rapid divergence in SR lengths is hypothesized to contribute to reproductive incompatibilities. However, the selective forces driving rapid divergence in SR length and the implications for speciation are still not clear. In this study, we use two closely related *Drosophila* species, *D. americana* and *D. lummei,* to investigate the role of SR length as a reproductive barrier and to identify the genetic basis of the difference in SR length. There is a significant difference in both SR and sperm length between the two species; *D. lummei* has much longer seminal receptacles (~10 mm) and sperm than *D. americana* (~7 mm). We use whole chromosome mapping and quantitative trait locus (QTL) mapping to identify the genetic basis underlying this difference. Whole chromosome mapping indicates that the major effect locus is located on the fused chromosomes 2 and 3. We also examine the correlation between SR length and the degree of reproductive isolation using *americana-lummei* hybrids. Our results shed light on the interplay between sexual selection and speciation, and the role of rapid divergence in post-copulatory traits in generating reproductive isolation.

350F **Genomics of experimentally-evolved postponed reproduction in *Drosophila melanogaster*** Giovanni A Crestani[1], Alma Karen Gamboa Walsh[2], Alejandro Moran[2], Hannah S. Dugo[1], Parvin Shahrestani[2], Molly K Burke[1] [1]Integrative Biology, Oregon State University, [2]California State University, Fullerton

Evolve and resequence (E&R) experiments with model organisms have the potential to reveal the genetics underlying complex traits and investigate the molecular dynamics of adaptation. Here, we sequence the genomes of Drosophila melanogaster populations experimentally-evolved for postponed reproduction for over 20 generations. These populations, maintained on a 70-day generation cycle, rapidly evolved a suite of life-history phenotypes that differ from control populations kept on a standard 14-day generation cycle. Experimental populations live significantly longer than controls and exhibit many correlated phenotypes. The experimental system we used involves four selection treatments, each consisting of five replicate populations. The two selection treatments on a 70-day (O-type) cycle differ in their recent evolutionary history: the OB treatment originated from populations that had always been reared on a 14-day generation cycle, and the OBO treatment was derived from populations recently reverted from a 70-day to 14-day generation cycle. From those same ancestral populations, we derived two control treatments that maintain a 14-day (B-type) cycle: nB, which has always been on a 14-day cycle; and nBO, derived from the same populations that founded the OBO treatment. Each of those four selection treatments represents a unique combination of ancestral and contemporary selective pressures. By comparing the genomes of populations of the same selection treatment, but with different evolutionary histories, we can test hypotheses about the degree to which past evolution constrains future adaptation. Using pooled-population sequencing, we collected whole-genome allele frequency data from all populations at multiple time points over 20 generations of O-type selection. Our results show that allele frequencies are strongly predicted by selection treatment, but not by recent evolutionary history, revealing a remarkable level of evolutionary convergence within selection regimes. Additionally, our results support a model where adaptation for postponed reproduction has a polygenic basis, and occurs rapidly via shifts in standing genetic variation across the genome.

351F **Teaching a Centromere to Drive** Nicolas D Lee[1], Andrea Carroll[2], Bhavatharini Kasinathan[3], Aida de la Cruz[1], Harmit S Malik[1] [1]Fred Hutch Cancer Center, [2]Stanford Medicine, [3]UW Medicine

In most eukaryotes, the essential process of chromosome segregation is orchestrated by repetitive DNA sequences known as centromeres. Centromeric histone H3 (CenH3) histone variants bind specifically to centromeric DNA and recruit the molecular machinery necessary for faithful cell division. CenH3 loss or overexpression can lead to centromere dysfunction, which is associated with genome instability. Despite their essential function, both centromeric DNA and centromeric proteins (including CenH3) evolve rapidly. The 'centromere-drive model' posits that this unexpected rapid evolution results from competition during oogenesis between different meiotic chromosomes for inclusion in the egg instead of polar bodies. A vital principle of this model is that excess recruitment of CenH3 proteins to repetitive centromeric DNA confers a meiotic advantage. Past studies with heterozygous mouse strains containing centromeres with different lengths of repetitive DNA strongly support the model that higher CenH3 recruitment may lead to female meiotic success. However, these chromosomes also differ in many other sequence aspects, which may influence meiotic transmission. To overcome these difficulties, we propose to engineer and test a new approach to recruit CenH3 to repetitive DNA in the model fruit fly species *Drosophila melanogaster* (*D. mel*). We will utilize a novel tool for generating targeted centromeres in *D. mel* using a fusion of catalytically dead Cas9 (dCas9) and the *D. mel* CenH3, also known as Cid. With this tool, we plan to induce a chromosome with an expanded centromere by using gRNAs to target the dCas9-Cid fusion to the pericentromeric satellite repeat of a single chromosome, mimicking the rapid centromere expansions that can occur due to unequal crossing over in meiosis. These studies will evaluate the fitness consequences of excess CenH3 recruitment in cell division and unambiguously test the centromere-drive model.

352F **Experimental evolution of fitness tradeoffs associated with spatially-varying balancing selection in herbivorous drosophilid flies** Diler Haji[1], Andy Gloss[2], Joy Bergelson[2], Julien Ayroles[3,4], Noah Whiteman[1,5] [1]Integrative Biology, University of California, Berkeley, [2]Center for Genomics and Systems Biology, New York University, [3]Ecology & Evolutionary Biology, Princeton University, [4]Lewis-Sigler Institute for Integrative Genomics, Princeton University, [5]Molecular & Cell Biology, University of California, Berkeley

Natural drosophilid populations harbor unexpectedly high genetic diversity, potentially maintained through both temporal and spatial variation in selection pressures. While temporal variation is well studied, spatial variation's role remains understudied despite its greater potential for maintaining genetic polymorphisms. The Levene Model (1953) proposes that spatially-varying selection can maintain alternative genotypes within populations through divergent selection across environmental niches. We conducted the first genome-wide test of this model using Scaptomyza flava, a drosophilid fly that oviposits in leaves of two sympatric mustard species, Barbarea vulgaris and Turritis glabra, in New Hampshire fields. These flies develop as leaf miners, mate randomly as adults, and have no apparent assortative mating by host plant, making them ideal for testing the Levene Model. We maintained flies from wild populations for ten generations under three experimentally-replicated conditions: B. vulgaris only, T. glabra only, and both species combined. Single-host populations showed local adaptation, producing 8% more offspring on their evolved host compared to the alternative. We identified a repeatable, polygenic basis for host adaptation, with selection intensities of 1-18% per generation. Directional allele frequency changes and parallel evolution across replicates suggest fitness tradeoffs mediated by antagonistically pleiotropic genetic variants. These findings demonstrate spatial variation's role in maintaining genetic diversity in S. flava and support the Levene Model's predictions.

353F **The spatiotemporal evolution of apical extracellular matrix (aECM) in the rapidly diversifying Drosophila genitalia.** Catarina Colmatti Bromatti[1], Donya Shodja[2], Ben Vincent[3], Mark J Rebeiz[4] [1]Biological Sciences, University of Pittsburgh, [2]George Washington University/University of Pittsburgh, [3]California State University Los Angeles, [4]University of Pittsburgh

The evolution of morphological novelties is a major focus of evolutionary developmental biology. Changes in the expression of transcription factors are often critical in explaining how new structures deploy genetic networks to achieve their unique morphological features. However, we have few examples describing how terminal effectors, which directly alter cellular behavior become deployed in new tissues. Here, I use *Drosophila melanogaster* as a model organism to study the origin of the novel genital structure called the posterior lobe. Previous work has found that accumulation of the apical extracellular matrix (aECM) in and around the posterior lobe during its development is correlated with its origin. The cellular effector Dumpy, one of the main components of the aECM, is expressed in the posterior lobe, and disruption of *dumpy* causes the near ablation of the posterior lobe. To trace how the aECM evolved functions in the development of the posterior lobe, I examined genital expression patterns of several loci that interact with Dumpy in other tissues using the hybridization chain reaction (HCR). Additionally, to examine how the expression of Dumpy expanded in the posterior lobe, I mapped the regulatory region of *dumpy,* identifying multiple genital elements that recapitulate its complete pattern of expression in the genitalia. Notably, each genital structure which expresses *dumpy* including the posterior lobe is driven by multiple separate enhancers, hinting at the presence of a robust system underlying both new and old structures. My results highlight how terminal effectors may be governed by to a highly complex, redundant regulatory architecture.

354F **Pleiotropy or Linkage: The genetic basis of a correlation between color and behavior** Sarah N Ruckman, Kimberly A Hughes Biological Science, Florida State University

One long standing question in evolutionary biology is whether single genes that control multiple traits (pleiotropy) result in limitations on adaptive evolution. If so, then our ability to predict adaptation (e.g., in the face of changing environments) is compromised. We are using a much-discussed correlation between body coloration and behavior as a system to address this question. Aggression and pigmentation are hypothesized to be genetically correlated in many organisms because the same biochemical pathways (e.g., dopamine synthesis) are used in pigment production and the synthesis of molecules that modulate behavior, including aggression. We employed a selection experiment in two species of fruit flies, *Drosophila melanogaster* and *D. simulans* to test this hypothesis. To test for genetic correlation between color and behavior, we selected for darker (and lighter) body color, relative to controls, in replicate populations. We measured thoracic pigmentation and behavior during 15 generations of selection and 5 generations of relaxed selection. In both species, dark-selected male flies evolved to be more aggressive, indicating a genetic correlation between color and behavior. In both species, dark-selected females were not more aggressive. In both species and both sexes, dark-selected flies were more active, indicating a genetic correlation between color and activity level. To distinguish between pleiotropy and linkage disequilibrium as a cause of correlated evolution (and potential evolutionary constraint), we will next identify candidate genes using RNA-Seq and test for pleiotropic effects using RNAi experiments.

355F **A regulatory locus contains both a seasonal and clinal SNP that determines embryonic heat tolerance in *Drosophila melanogaster*** Joaquin C. B. Nunez[1], Sumaetee Tangwancharoen[2], Kylie M. Finnegan[3], Brent L. Lockwood[1] [1]Biology, University of Vermont, [2]Chulalongkorn University, [3]Harvard University

Despite decades of research into the genetics and physiology of responses to environmental change, we know relatively little about the genetics of environmentally influenced traits across the life cycle of species with complex life histories. Previously, we reported that patterns of natural variation in heat tolerance are life-stage specific in *Drosophila melanogaster*, suggesting that thermal selection predominantly targets the early embryonic life stage. Here, we used advanced introgression and pooled whole-genome resequencing to map the genomic basis of enhanced embryonic heat tolerance in a neotropical line of *D. melanogaster*. We discovered two loci that mapped to regions on chromosomes 2R and X that were consistently targets of 16 generations of thermal selection across 6 replicate introgressions. Further comparison of the alleles in these two genomic regions to published datasets of naturally occurring allelic variation across North America and Europe (using the DEST dataset) revealed that one embryonic heat tolerance SNP on chromosome 2R exhibited both clinal and seasonal patterns of variation. Moreover, alleles at this locus were significantly correlated to environmental variability across space and time in average humidity, average precipitation, and variance of temperature. The SNP lies in the regulatory region of a gene that encodes an endopeptidase, and individuals with different alleles at this locus exhibited disparate gene expression responses of this gene to heat stress. Overall, our results suggest that loci that influence embryonic heat tolerance are under selection in nature. Notably, embryonic heat tolerance was not genetically correlated to adult heat tolerance. Thus, embryonic heat tolerance is an ecologically relevant trait that has a distinct genetic basis. Our study extends previous work in developmental genetics of *Drosophila* by characterizing the genomics of an ecologically relevant developmental trait in natural populations.

356F **Comparative Genomics and the Evolution of Immune Genes in *Drosophila*** Pankaj Dhakad IEE, University of Edinburgh

Accurate prediction of genomic features lays the foundation for evolutionary analyses. In this study, we employed the Comparative Annotation Toolkit (CAT) pipeline, supplemented with Braker3, to annotate 304 Drosophila genomes. CAT offers a comprehensive approach, integrating transcript projection, transcriptome, and proteome alignments, along with simultaneous gene-finding. Nevertheless, CAT's efficacy in identifying species-specific genes appears limited in the absence of RNAseq data or high-quality reference annotations. To address this limitation, we complemented CAT annotations with those generated by braker3, leading to substantial improvements in annotations. To assess annotation quality across these Drosophila species, we employed phylogenetic mixed models to infer phylogenetic effects on gene number and length, along with the effect of RNAseq availability and distance from a reference species.

With these annotations, we investigated the evolutionary dynamics of Drosophila immune genes. Using CAFE5, we estimated gene turnover rates (λ) in immune genes and compared them to size- and location-matched control genes. Immune genes exhibited significantly higher turnover rates, potentially driven by selective pressures to adapt to diverse pathogens and environmental challenges. Surprisingly, although immune genes did not show significantly different nonsynonymous-to-synonymous substitution rates (dN/dS) compared to controls, dN/dS values varied across immune functional classes, indicating selective pressure may act more on genes directly interacting with pathogens.

Overall, our study demonstrates the value of comparative annotation approaches for accurate genome annotation. In addition, our gene turnover and dN/dS analysis offer insights into how immune genes evolve in response to pathogen-driven selection pressure.

357F **Genetic consequences of a host-DNA virus interaction: *Drosophila innubila* nudivirus (DiNV)** Taiye S. Adewumi, Robert Unckless Molecular Biosciences, University of Kansas

Drosophila innubila nudivirus (DiNV) is a naturally occurring double-stranded DNA virus that exhibits high virulence in members of the quinaria group. The ability of DNA viruses in invertebrates to adapt to diverse host species—and the potential virulence in whole organisms—along with the genetic consequences of these adaptations, remains largely unexplored.

Here, we explore DiNV adaptation to *Drosophila melanogaster* cell line (S2 cells), hypothesizing that genetic mutation drive adaptation to processes such as viral entry, replication, or assembly. We injected (thoracic) *D. melanogaster* flies with adapted or naïve DiNV into either wild type or Myd88/IMD null mutants and monitored survival for 20 days. Additionally, viral DNA was extracted from both an ancestral DiNV passage from a *Drosophila innubila* cell line and an evolved passage in the S2 cell line (11 passages). Using short-read sequencing we compared found genetic differences between evolved and ancestral DiNV strains. Our results indicate that the virus passaged in S2 cells (evolved strain) demonstrates enhanced viral replication and infection ability in S2 cells compared to the ancestral strain. Additionally, we show that the evolved virus exhibits genotype-specific mortality in *D. melanogaster*. Genome sequencing of the virus identified potentially causative SNPs. The adaptation of DiNV to S2 cell lines demonstrates that DNA viruses in invertebrates can develop host-specific adaptations that affect virulence.

358F **Gene duplication captures morph-specific promoter usage in the evolution of aphid wing dimorphisms** Omid Saleh Ziabari[1], Fangzhou Liu[2], Kevin D Deem[3], Xiaomi Liu[3], Jennifer Brisson[3] [1]University of Pittsburgh, [2]Cornell University, [3]University of Rochester

Understanding how morphology evolves requires identifying the types of mutations that contribute to changes in development. We integrated comparative genomics and transcriptomics to reconstruct the evolution and regulation of *follistatin* paralogs in relation to the evolution of aphid winged and wingless morphs. We discover that different pea aphid *follistatin* duplicates play an essential molecular role in both the male and female wing dimorphisms, linking the genetic and environmental control of morph determination in each sex, respectively. We also find that an ancestral *follistatin* gene likely had multiple promoters, and that the *follistatin* duplicates that evolved wingless-specific expression retained only the ancestral wingless-specific promoter. Our work provides a roadmap for how alternative promoter usage and subsequent gene duplication can enable the evolution of animal form.

359F **Functional validation of tetrodotoxin and insecticide resistance mutations in *Drosophila* reveals patterns of cross-resistance and fitness trade-offs** Nitin Vincent, Stephan Espinosa, Kayla Miskovsky, Paddy Sullivan, Michael E Pfrender Biological Sciences, University of Notre Dame

Natural populations are often exposed to complex mixtures of toxic compounds of natural and anthropogenic origins. The toxicity of naturally occurring Tetrodotoxin (TTX) and many commonly used insecticides is due to their effect on the performance of Voltage-gated Sodium Channels (VGSCs). Mutations in VGSCs can reduce the toxicity of these compounds, however, the correlated effect of mutations that have a large effect on susceptibility to one compound on susceptibility to other compounds is not well known. We are using Drosophila to study these correlated effects of VGSC mutations since they possess a single VGSC gene and it is possible to introduce specific amino acid substitutions into the coding sequence. We acquired Drosophila lines with VGSC mutations and compared mutant and wild-type Drosophila populations for various phenotypes of insecticide and TTX resistance including mortality, negative geotaxis speed, larval crawling speed and time to eclosion. We also analyzed several of these phenotypes between mutant and wild type flies in the absence of toxin to determine any potential fitness costs conferred by VGSC mutations. We found significant differences between wild type and mutant flies in presence of insecticides and TTX, and costs to locomotive ability in the absence of toxin in the mutant flies. Moreover, we found varying overlap between insecticide-resistance and TTX-resistance phenotypes. These findings corroborate our preliminary results and suggest that there is an interaction between insecticide and TTX resistance, and significant fitness costs to toxin resistance in the absence of toxins.

360F **Contribution of locally adapted variation to adaptive potential in experimental populations of *Drosophila melanogaster*** Jamie Freeman, John Pool Genetics, University of Wisconsin- Madison

Genetic diversity is central to the ability of a population to adapt to its environment, but neutral genetic diversity does not always have a simple relationship with fitness. Local adaptation of populations to distinct environments provides a potential reservoir of fitness relevant variation. Experimental cages comprised of *Drosophila melanogaster* from Zambia, highland Ethiopia, and France were founded with equal numbers of inbred lines from one, two, or all three of the source populations. In separate experiments, these 18 cages were selected over 10 generations against two distinct selection pressures: survival on a toxic food source dosed with a detergent and survival in a wounding assay. Survival and population size metrics were measured over the 10 generations of selection. For the detergent feeding assay, the cages founded with lines from a single geographic source had lower population sizes over the course of the experiment than cages founded with two or three geographic sources.

361F **Examining the impacts of competition on host usage in a generalist herbivore** Kendra Casse, Clare Scott Chialvo Biology, Appalachian State University

In the natural world, species experience competition which can directly impact their ability to survive and reproduce. Understanding the impacts of competition on host usage is an important question for ecologists and evolutionary biologists. One example of this can be seen with *Drosophila tripunctata*, a generalist that feeds on both fruit and mushrooms, including toxic mushrooms. Despite representing a small portion of its acceptable hosts, *Drosophila tripunctata* can feed and develop on toxic deathcap mushrooms that would kill most eukaryotic organisms. Furthermore, females exhibit a strong preference for laying eggs on either fruit or mushrooms, but it is unknown why females may opt for toxic mushroom hosts. Little is known about how competition may impact female host preference of *D. tripunctata* for toxic and edible hosts alike. For this study, we will use competition assays to evaluate whether competition for egg laying sites influence host preference in *Drosophila tripunctata*. Specifically, we aim to determine if the presence of interspecific and/or intraspecific competitors cause *D. tripunctata* to preferentially use toxic mushrooms as hosts. The results of our study will help elucidate how both interspecific and intraspecific competition impacts host usage and how it helps maintain the ability to use toxic hosts. Thus, these results will help to expand our knowledge of the role of competition in host usage in generalist species.

362F **Characterizing the effects of host preference on a generalist species** Haley Martin[1], Grace Kropelin[2], Clare Scott Chialvo[1] [1]Appalachian State University, [2]Biology, Appalachian State University

Understanding how host usage impacts the evolution of herbivorous insects is an active area of study. Species with broad host ranges and populations that exhibit distinct preferences may undergo sympatric speciation. However, it is not clear how likely these types of herbivore-host interactions are to initiate the process of sympatric speciation. Fruit flies in the genus *Drosophila* exhibit a wide range of feeding behaviors. One species, *Drosophila tripunctata*, is a generalist that feeds on fruit and mushrooms, including highly toxic Death Cap mushrooms that kill most eukaryotic organisms. Furthermore, female *D. tripunctata* show distinct preferences when choosing an egg laying site between fruit and mushroom. This is a good model to try to understand the processes by which sympatric speciation might occur. The goal of this study is to characterize how the relationship between host choice and toxin tolerance has impacted the evolution of *D. tripunctata* and assess whether sympatric speciation is occuring. To accomplish this we will use oviposition and toxin tolerance assays to quantify variation in both traits. The results of our assays will help to expand our understanding of these traits and assess a population for the possibility of sympatric speciation. Thus, our study will help provide more information on how host usage in a generalist impacts the evolution of a species.

363F **Aging and metabolomics: Insights from experimentally evolved *Drosophila melanogaster*** David L Hubert[1], Kenneth Arnold[2], Zachary Greenspan[2], Benjamin Harrison[3], Mark A Phillips[4] [1]Integrative Biology, Oregon State University, [2]University of California, Irvine, [3]University of Washington, [4]Oregon State University

Experimental evolution studies that feature selection on life-history characters are a proven approach for studying the evolution of aging and variation in rates of senescence. Recently, the incorporation of genomic and transcriptomic approaches into this framework has led to the identification of hundreds of genes associated with different aging patterns. However, our understanding of the specific molecular mechanisms underlying these aging patterns remains limited. Here, we incorporated extensive metabolomic profiling into this framework to generate mechanistic insights into aging patterns in *Drosophila melanogaster*. Specifically, we characterized metabolomic change over adult lifespan associated with accelerated aging in populations of *D. melanogaster* under selection for early reproduction compared to their controls. Using these data we: i) evaluated the evolutionary repeatability across the metabolome; ii) evaluated the value of the metabolome as a predictor of “biological age” in this system; and iii) identified specific metabolites associated with accelerated aging. Metabolomic analysis revealed that generations of selection for early reproduction resulted in highly repeatable alterations to the metabolome. Specifically, we find clusters of metabolites that are associated with aging, adding new insights into the metabolites that may be driving the accelerated aging phenotype.

364F **The role of behavioral adaptation to high sugar diets in *Drosophila melanogaster*** Austin D Vick, Mark A Phillips Integrative Biology, Oregon State University

Type II diabetes mellitus (T2DM) is a complex metabolic disorder primarily induced by a high-sugar diet (HSD). In brain tissue, T2DM contributes to neurodegeneration through the accumulation of cellular debris and metabolic byproducts, leading to a decline in neural function. To better understand the impacts of T2DM on neuroplasticity and behavior, several studies have utilized Drosophila melanogaster as a model organism. These studies have provided insights into the effects of a high-sugar diet on brain health and behavior; however, they have largely been conducted with inbred populations. This limitation may impact the generalizability of findings, as genetic diversity can influence baseline behaviors and potentially alter responses to dietary interventions.This research aims to address this gap by utilizing an outbred population of Drosophila, allowing for a broader range of behavioral and metabolic responses. Additionally, previous findings suggest that Drosophila subjected to increased physical activity and exercise can mitigate the adverse effects associated with a high-sugar diet. Building on this knowledge, this study will use a variety of behavioral assays and metabolomic sequencing techniques to elucidate the underlying mechanisms that contribute to neuroplasticity in Drosophila on a high-sugar diet.A particular focus of this work will be on neurotransmitters, which play crucial roles in regulating glucose levels, circadian rhythms, locomotor functions, and overall neuroactivity. By selecting for active behaviors—characterized by heightened locomotor activity, elevated octopamine levels, and increased overall activity—this study will investigate whether a high baseline of physical activity can solely counteract the negative neurological and metabolic impacts of a high-sugar diet in an outbred population. Given the established link between increased activity and improved outcomes in populations exposed to high-sugar diets, we hypothesize that promoting active behavior will provide similar protective effects in an outbred Drosophila population after several generations of adaptation.

365F **Adaptive mechanism to high-sugar diet in *Drosophila melanogaster*: A model for diabetes resistance** Elmira Ahmadian[1], Mark A Phillips[2] [1]Oregon State University, [2]Integrative Biology, Oregon State University

The global rise in diabetes prevalence demands innovative research models to unravel the genetic and physiological mechanisms underpinning susceptibility and resistance. Drosophila melanogaster, with its genetic and metabolic similarities to humans, serves as an ideal model for studying complex metabolic disorders such as type 2 diabetes. This project aims to explore the adaptive mechanisms of fruit fly populations subjected to a high-sugar diet (HSD) over multiple generations, creating a model for diabetes resistance. In the initial phase of this research, we exposed control and treatment groups of Drosophila to either a standard diet or an HSD and assessed lifespan, mortality rates, and reproductive output to characterize phenotypic responses. Preliminary results indicate that HSD-fed flies exhibit reduced median lifespan and higher mortality rates, particularly in males. Furthermore, reproductive assays reveal diminished egg production in HSD females, suggesting an adverse impact on fecundity. These findings support previous research linking HSD to insulin resistance and metabolic dysfunction in Drosophila. Ongoing analyses include quantifying glucose and triglyceride levels to track physiological markers of diabetes-like conditions in response to HSD. These measurements will provide insights into the metabolic adjustments associated with HSD adaptation over successive generations. Our hypothesis posits that adaptive physiological changes will mitigate the fitness costs associated with HSD, potentially revealing key pathways for diabetes resistance. This study lays the groundwork for advanced molecular analysis in subsequent research phases, including transcriptomic and metabolomic profiling. Ultimately, our findings will enhance the understanding of diabetes resistance and pave the way for identifying biomarkers for susceptibility, with broader implications for managing metabolic disorders.

366F **High-Sugar Diet Effects on Insulin-Producing Cells-Ablated Fly and Genetic and Dietary Interactions in Metabolic Regulation** Yuyan Chen, Xuan Zhuang University of Arkansas Fayetteville

Metabolic disorders, including diabetes, can be affected by genetic and dietary factors. Prior research created a genetically modified fly line with ablation of insulin-producing cells (IPCs), resulting in compromised insulin signaling and changed glucose balance. Moreover, flies subjected to a high-sugar diet (HSD) exhibit metabolic disturbances, including elevated glucose levels and lipid accumulation. Notably, certain characteristics, including metabolic traits and fecundity, exhibit similar trends in IPCs-ablated flies and HSD-fed flies, whereas they display contrasting trends in dilp2 expression and insulin resistance and sensitivity. Nonetheless, the interaction between genes involved in the insulin signaling pathway and dietary conditions remains ambiguous. Therefore, this project aims to investigate the connections between IPCs-ablation intervention and sugar diet intervention across several features. We provide high and low sucrose diets to IPC-ablated adult flies for experimental purposes. Following a high-sugar diet and IPC-ablation intervention, various characteristics, including metabolite levels and gene expression, were assessed. Initial findings indicate significant potential interactions between the IPC-ablation intervention and the sugar diet intervention in several traits such as glycogen, glucose, triglycerides, and dilp2 expression levels. Moreover, we will perform RNA sequencing to identify the genes with differential expressions among each tested group. Further conclusions will be validated through additional tests and rigorous statistical analyses to enhance the robustness and reliability of the findings.

367S **The genetic diversity and population structure of locally-adapted chickens (gallus gallus domesticus) from Nigeria** Opeyemi Oladejo[1], Saidu Oseni[2], Adeniyi C. Adeola[3] [1]Bowen University, Iwo, Vigeria, [2]Obafemi Awolowo University, Ile-Ife, Nigeria, [3]State Key Laboratory of Genetic Resources & Evolution, Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming, Yunnan, China

Haplotype and nucleotide diversity are two important separate indices for assessing population polymorphism and genetic differentiation.

Genetic information about chickens is important for making decision on conservation, improvement and sustainable in their various production environments.

Justification: The information on the improved breeds; FUNAAB-Alpha (FAC) and Noiler (NC) with indigenous ecotypes; Fulani (FEC) and Yoruba (YEC) of Nigeria locally-adapted chicken (NLAC) is least available in the literature especially using LEI0258 avian Major Histocompatibility Complex (MHC) molecular markers.

Objectives: The objective of this study was to assess the genetic diversity and phylogenetic population structure in four different NLAC genotypes using MHC genetic markers as well as compare with chickens from other countries.

Methodology: Blood samples were randomly collected from about 200 NLACs, stored in ethylenediamine-tetraacetic-acid (EDTA) tubes as well as on Flinders-Technology-Associates (FTA) cards for Deoxyribonucleic Acid (DNA) extraction, sanger-sequencing and bioinformatics. For genetic variation and population structure of the MHC region of NLAC, network 10.1.0 software was employed. The FASTA files of MHC-linked LEI0258 sequences of chickens from five other countries (namely Ethiopia, Tanzania, China, Vietman and North America) were also stored and utilized. These sequences were downloaded from National Centre for Biotechnology Institute (NCBI) (n=137) merged with NLACs sequences (n=172). These LEI0258 sequences (N= 309) were aligned (Clustal W) and use to generate haplotypes.

Results: The population structure of the NLAC using analysis of molecular variance (AMOVA) indicated that NC (86%) had the highest haplotype diversity and FEC (74%) had the lowest; while YEC had the highest (8.2%) and FEC the least (2.1%) in nucleotide diversity. The observed high haplotype diversity values across all the chicken populations showed uniqueness and diversity of each of the NLAC populations while low nucleotide diversity observed however, indicated high level of relatedness or low variability within each of the populations using LEI0258 MHC-linked molecular marker.

Conclusions: The admixture of populations in the six countries indicates mixed genetic backgrounds of the chickens as well as common source of origin.

368S **Accelerating chromosome evolution using the *Drosophila melanogaster* B chromosome** Edward S Russell, Stacey L. Hanlon Molecular and Cell Biology, University of Connecticut

The genome is in constant flux as it endures frequent changes to its composition and chromosome complement. An example of such a change is the appearance of B chromosomes, which are nonessential accessory chromosomes that are often maintained in a population through mechanisms that “drive” its biased inheritance. Though these drive mechanisms can be complex, the current model for B chromosome evolution begins with a fragment that originated from an essential chromosome and lacks the ability to bias its inheritance. As this “proto-B” matures, it is thought to accumulate unique sequences and develop a successful drive mechanism. Though several established B chromosome systems support this model, the stepwise details of how a proto-B can acquire its own sequence and evolve into a mature B chromosome remains elusive. To investigate the evolution of a B chromosome, we are using the B chromosomes recently discovered in a single laboratory stock of *Drosophila melanogaster*. Unlike traditional rye and maize B chromosome models, which are millions of years old, the *D. melanogaster* B chromosome is a proto-B that is a mere 20 years old. It was recently derived from an essential chromosome, carries no known genes, does not appear to have any unique sequence elements, and is maintained in the stock through a host-derived drive mechanism rather than its own. To accelerate the maturation of this B chromosome, we are inducing the mobilization of a modified P-element to determine if it is able to transpose into the B chromosome and be expressed. Our goal is to illustrate that the sequence from essential chromosomes can successfully transpose into, and be expressed by, a nonessential B chromosome. The presence of the P element on the B chromosome would also provide a landing site for the insertion of other sequence elements. By placing transgenic constructs onto the B chromosome that carry unique sequence elements or promote B chromosome drive, we aim to further test the model of B chromosome evolution. This research will begin to bridge the gap in our understanding of how newly formed B chromosomes can develop their own drive systems and acquire unique sequences as they mature, as well as interrogate the mechanisms that govern novel chromosome genesis and evolution.

369S **Regulation and evolution of a novel gene that specifies a reproductive structure in *Drosophila*** Ashley Bentz, Md Golam Azom, John P. Masly The University of Oklahoma

The epandrial posterior lobes (ePLs) are novel male genitalia structures found among the four sister species of the *Drosophila melanogaster* species complex (*D. melanogaster, D. mauritiana, D. simulans, and D. sechellia*). These structures are necessary for successful copulation and have evolved striking differences in size and shape among these species. Through genetic mapping and tests of genes that appear to be strong candidates for specifying ePL differences, we identified a gene we have named *Goldilocks* (*Glds*) that specifies ePL size. To understand more about this gene's role in development, it is important to examine its interactions with other genes. *Pox neuro* encodes a transcription factor that is necessary for ePL development in *D. melanogaster*. Using a *Poxn* null allele, we performed *in situ* hybridization against *Glds* to identify whether *Poxn* regulates *Glds* during ePL development. Our results show that *Glds* expression is unchanged in *Poxn* null males compared to the wildtype, which indicates that *Glds* is not regulated as a consequence of *Poxn* expression. We also characterized *Glds* expression in the developing male genitalia in *D. yakuba* and *D. santomea*, two species that lack ePLs. Our results show that *Glds* localizes broadly within their genital primordia, which suggests that *Glds* expression predates the evolution of the ePL and its function might have been co-opted into the ePL gene regulatory network. To further understand the evolution of *Glds*, we identified homologs in 43 *Drosophila, Bactrocera, Musca,* and *Episyrphus* species. We analyzed these homologs for patterns of molecular evolution and found they all contain a signal peptide sequence at the N-terminus, similar to the *D. melanogaster* complex species. The conservation of this signal peptide may suggest that *Glds* broadly functions as a signaling molecule in these species and has only recently been recruited to direct ePL development.

370S **Developmental causes of the evolution of mutation rates** Paco Majic EMBL Heidelberg

Mutation rates are an evolvable feature. A widely accepted view of how mutation rates evolve involves 1) the evolution of mutator alleles that affects the efficacy of DNA repair mechanisms and 2) a selective pressure at the individual level to reduce the burden of deleterious mutations. I will here present an alternative view of how mutation rates evolve which is based on the evolution of developmental features. Under this view, mutation rates can evolve not as a result of a selective pressure but rather neutrally, as the byproduct of changes in developmental parameters. I will present theoretical and experimental results supporting this hypothesis, together with examples of how considering the developmental causes of mutation rate evolution could help explain well-known micro- and macroevolutionary trends.

371S **Evidence that the *Stellate* array of *Drosophila melanogaster* is an active meiotic drive system** Benjamin K McCormick, Daniel A Barbash, Andrew G Clark Molecular Biology & Genetics, Cornell University

Meiotic drivers are selfish elements that bias their own transmission such that they are overrepresented among the functional gametes produced. The selective costs imposed by drivers on their hosts may trigger intragenomic conflict, leading to the emergence of suppressors and subsequent arms race dynamics between the driver and suppressors. *Stellate* (*Ste*) is a tandemly arrayed multicopy gene, and we find that its copy number ranges from 3 to almost 300 among X chromosomes of *Drosophila melanogaster* from the Global Diversity Lines. In wild-type animals, *Ste* expression is usually suppressed by homologous piRNAs produced from the *Suppressor of Stellate* (*Su(Ste)*) array on the Y chromosome. Derepression of *Ste* in the absence of *Su(Ste)* results in the formation of proteinaceous crystals in spermatocytes, chromatin compaction defects, reductions in fertility, and female-biased sex ratios owing to the under-recovery of Y-bearing sperm. Despite extensive study, the function of the *Stellate* array and evolutionary significance of its persistence in the genome have remained elusive. It has been suggested to be a now-inactive relic of an ancient meiotic drive system, though this idea has not been rigorously tested. We established crosses between females with high *Ste* copy number X chromosomes and males whose Y chromosome harbored low copy number *Su(Ste)* arrays and found that the male progeny displayed non-Mendelian sex chromosome transmission. Results are consistent with *Stellate* being an active drive system, with high copy number alleles potentially overcoming suppression in a dose-dependent manner.

372S **The effects of the post-mating immune response in Drosophila on Female fecundity.** Kross E McClinton, Yasir H Ahmed-Braimah Syracuse University

Understanding the molecular processes that govern reproductive success is a fundamental goal of evolutionary genetics because reproductive fitness is a key determinant of evolutionary trajectories. One of the pervasive observations in reproductive biology is that—in addition to initiating a cascade of regulatory events that activate the reproductive cycle—females launch an immune response after mating. It is thought that this immune response to mating is a preemptive defense mechanism in anticipation of potential pathogen infiltration during copulation, but recent evidence shows that the magnitude of this immune response is strongly modulated by paternal genotype. Specifically, work in *Drosophila* and other species has shown that interspecies mating results in a heightened immune response. Moreover, recent results from the Ahmed-Braimah Lab shows that males that transfer reduced seminal fluid and sperm during copulation induce a dampened immune response in females post-mating. These observations suggest that the immune response may play additional roles in mated females that are not strictly concordant with the preemptive strike hypothesis. Here I will examine the effect of the localized immune response on (1) reproductive success and (2) postmating gene expression by deactivating the immune response in *D. melanogaster* females. By performing a set of female fertility assays and postmating gene expression analysis I will identify the significance of the immune response in the female reproductive tract and directly address the hypothesis that the immune response plays a role in females' reproductive success.

373S **Origin story of a Y chromosome** Taylor D Conway[1], Ryan Bracewell[2], Grace Lee[3], Garnet Phinney[3], Robert L. Unckless[1] [1]EEB, University of Kansas, [2]Indiana University, [3]University of California - Irvine

The Y chromosome in many species is degenerative and gene-poor due to the lack of recombination and accumulation of deleterious mutations, yet it remains essential for male fertility in most *Drosophila* species. *Drosophila affinis*, however, is unique among *Drosophila* species in that it is one of the few can produce fertile males without a Y chromosome (XO males). This study investigates the origin and evolutionary dynamics of the *D. affinis* Y chromosome. By employing Pacbio hifi sequencing on male and female genomes, we identified Y-linked sequences and performed synteny analysis *D. pseudoobscura* and *D. melanogaster* to help trace Y chromosome origins. Results are expected to clarify whether the Y in *D. affinis* originated from the Muller D element and to highlight distinctions between the Y chromosomes of *D. affinis* and other *Drosophila* species.

374S **Mitonuclear compatibility: How genotype shapes mate selection in *Drosophila melanogaster*** Camille P Brown[1], Leah Darwin[2], Yevgeniy Raynes[1], Rebecca Bachtel[1], Faye Lemieux[1], David Rand[1] [1]Department of Ecology, Evolution, and Organismal Biology, Brown University, [2]Center for Computational Molecular Biology, Brown University

As the primary producers of cellular energy and sites of critical metabolic and signaling processes, mitochondria are essential to the survival of most eukaryotic cells. These organelles, once free-living eubacteria, contain their own independently replicating genome, yet are dependent on over 1000 imported nuclear gene products to properly function. The two genomes evolve together, with mitochondrial adaptation being driven by selection in females of a species. Due to the intimacy of mitonuclear interactions, incompatibilities between the genomes have been shown to reduce the mitochondria's metabolic ability in cyto-nuclear hybrids, and in plants are often implicated in hybrid breakdown. Sexual selection theory predicts that females should choose mates with 'good genes' that will benefit their offspring, and that males with 'good genes' can compete successfully for access to mates. The influence of mitonuclear interactions on organism viability has prompted the development of a 'mitonuclear matching hypothesis' that extends sexual selection theory to include female choice based on the compatibility of the female mitochondrial genome with the male nuclear genome. We use a *Drosophila melanogaster* system with introgressed mitochondrial (mt) genomes, two 'native' mtDNAs from *D. melanogaster* strains (Beijing and Zimbabwe), and a third 'foreign' mtDNA from *D. yakuba*. Each of these three mtDNAs were placed on two common nuclear backgrounds, *D. melanogaster OregonR* or *DGRP375*. Pairwise mate choice assays were used to test the hypothesis that individuals preferred 'native' mtDNAs over the 'foreign' *D. yakuba* mtDNA. The choice experiments included tests with one female and two males , and one male and two females, of each of the respective genotypes. Recording courtship, receptivity, and copulatory behaviors provides measures of male performance and both male and female preference that indicate how mitonuclear compatibility may be used as a criterion in the evaluation of potential mates. Females showed a significant preference for males of the *DGRP375* nuclear background, regardless of their own lineage. However, we observed no significant preference for either of the mtDNA's on both of the nuclear backgrounds, contrary to the predictions of the mitonuclear matching hypothesis.

375S **Discerning between convergence and shared ancestry in a genital novelty** Christopher K Darfoor, Mark Rebeiz Biological Sciences, University of Pittsburgh

The emergence of novel morphological traits is a challenging problem that requires examination at varying evolutionary depths. For very old novelties, which have been the target of previous studies, we expect that many of the initial steps in their evolution have been obscured by the loss of species through extinction. In contrast, in very young novelties, those intermediate steps may be preserved in the form of related species with highly diverse iterations of the novelty. Approximately 4 million years ago, relatively recently in evolutionary time, the posterior lobe emerged as a projection from the lateral plate of the common ancestor of *Drosophila melanogaster* and *Drosophila yakuba* and has since rapidly diversified. Surprisingly, we find that the montium group species *Drosophila auraria*, which was thought to have diverged from the *D. melanogaster* lineage before the posterior lobe evolved (approximately 23 million years ago), possesses a structure similar to the posterior lobe, hereafter referred to as the "pseudo-lobe." This raises questions about whether the posterior lobe and pseudo-lobe share a common origin or if they evolved convergently, where similar structures evolved independently. To answer this, I visualized tissue development in the posterior lobe and the pseudo-lobe to look for shared patterns of morphogenesis, which confirmed similar timing and cellular changes. In parallel, I have examined the expression of several highly expressed marker genes required for the posterior lobe in the *D. auraria* pseudo-lobe. These posterior lobe genes are not expressed in the *D. auraria* pseudo-lobe at the developmental time points I have investigated. Furthermore, in a survey of the montium group, I identified several species with small pseudo-lobe-like projections that are attached to different genital parent structures, suggesting that pseudo-lobes may have recurrently evolved in the montium clade or represent a structure with shared ancestry that has shifted in the position across evolution. The results of these analyses will provide critical insights into the evolution of novel morphologies.

376S **Variation in Susceptibility to Glyphosate-Based Herbicide Exposure Among DGRP Lines of *Drosophila*** Kelley Kelley[1], Katherine Bartels[1], Noelle Roddam[2], Bryan Gonzalez[1], Emily Wooten[3], Victoria Cordova[1], Becky Talyn[4] [1]Biology, California State University, [2]Human Genetics & Genetic Counseling, Keck Institute, [3]Biochemistry, California State University, [4]College of Natural Sciences, California State University

Glyphosate is the nominal active ingredient in Roundup, the most common herbicide used in agriculture. Glyphosate-based herbicides (GBHs) induce toxic behavioral, reproductive, and physiological effects on animals across taxonomic groups, including mortality, raising concerns about its environmental impact and potential implications for human health. Our research examines phenotypic variation in *Drosophila* sensitivity to GBH exposure among lines from the *Drosophila* Genetic Reference Panel (DGRP). These are a large set of highly inbred lines that are genetically well characterized, including single nucleotide polymorphisms, insertions, and deletions. Our methodology compares survival between exposed and unexposed flies over five paired trials for each line. For each trial, 5 male and 5 female newly-eclosed flies are placed in each treatment, 5g/L GBH in medium and organic medium. Mortality was recorded for males and females after 7 days to measure survival, and the presence of larvae or pupae recorded after 14 days to measure reproduction. Mortality rates of *Drosophila melanogaster* differ among DGRP lines when exposed to GBH treatments. Specifically, the relative survival (exposed / control) of the 36 lines tested so far ranges from 0.11 to 2.36, and relative reproduction ranges from 0 to 1. The variation in relative survival explains about ¼ of the variation in reproduction (R2=23.2%). Once we have completed mortality and reproduction measurements for at least 60 lines, we will compare phenotypic variability among lines to identify particular genetic variations that correlate with it. We hope to identify particular genes with alleles that differ between the lines most and least sensitive to Roundup exposure, both in terms of mortality and reproduction. We will combine the genetic results with those from differential transcriptome analysis of exposed and unexposed flies to identify target genes that might be involved in mechanisms of GBH toxicity. In addition to testing these target genes, our future research will further characterize the most susceptible and most resistant lines, and we will examine differences in composition of their gut and reproductive microbiome communities.

377S **The role of neuronal miRNAs in regulating behavioral states associated with female reproduction** Preston W Simpson[1], Amy Kwan[1,2], Nicole Leitner[1,3], Yehuda Ben-Shahar[1] [1]Biology, Washington University in St. Louis, [2]Grossman School of Medicine, New York University, [3]Molecular and Cell Biology, University of Arizona

The probability an individual will exhibit a specific behavioral trait is influenced by many factors, including their genetic background and environmental changes. Despite inherent variabilities associated with behavior, many animal species appear to have evolved mechanisms constraining phenotypic plasticity for at least some stereotypic, innate behaviors. Defined here as "behavioral states," this phenomenon is characterized by alternative suites of physiological traits that increase the likelihood an individual will exhibit stereotypic, species-specific behaviors in response to stimuli related to biological contexts (*e.g.*, age, sex, or reproductive state). Here we tested the hypothesis that animals regulate robustness of behavioral states through the action of microRNAs (miRNAs), and that these miRNAs function as key nodes in state-specific neuronal gene networks. In recent years, miRNAs have emerged as key homeostatic molecules, attenuating the activity of multiple protein-coding genes via diverse post-transcriptional mechanisms. In a previous screen, we identified several candidate miRNA genes that exhibit transcriptional regulation in association with nonreproductive division of labor in honey-bee colonies, a well-established model for temporal polyethism. Here we use *Drosophila* genetics to explore the role of phylogenetically conserved candidate miRNAs in the regulation of distinct behavioral states associated with female reproduction. Specifically, we focused our current analysis on *miR-210*, a miRNA highly conserved from worms to humans. Using genetic and transgenic manipulations, we show that *miR-210* plays a role in regulating the transition between female behavioral states in response to mating status (virgin-to-mated). In contrast to wild type mated females, mated homozygous *mir-210* null females appear to change their egg-laying behavior in the presence of males. These findings indicate that the transition from high male receptivity in virgin females to low male receptivity in mated females is incomplete in *miR-210* mutant females. Spatial characterization of *miR-210* expression in the female nervous system shows that it is highly enriched in subsets of the olfactory and gustatory chemosensory systems, as well as in specific subsets of photoreceptor cells in the visual system. Overall, our findings suggest that *miR-210* may regulate behavioral states by altering sensory response thresholds to state-specific stimuli in females.

378S **Ant odors alert flies to bury eggs** Todd Schlenke[1], Shaun Davis[2] [1]University of Arizona, [2]Hollins University

Ants are ubiquitous and consume insects at all life stages, presumably creating strong selective pressures 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 that insect mothers may have evolved oviposition strategies to minimize the ant predation risk to their offspring. 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 but we found that exposure to ants induces flies to bury their eggs deeply into the food. Buried eggs are markedly better at surviving ant foraging bouts than non-buried eggs, showing that this oviposition depth behavior is adaptive. Furthermore, this behavior is conserved across the genus Drosophila and is dependent on fly olfactory detection of ant presence. We identified two fly odorant receptors that are cooperatively required for ant detection. To further delineate the ant lineages to which flies respond we exposed flies to odor extracts from a diversity of ants and other insect species. Surprisingly, flies buried their eggs in response to the odors of nearly all hymenopterans tested but hardly any non-hymenopterans.

379S **Genomic Trajectories of Adaptation: Convergent Experimental Evolution in Drosophila** Kenneth R Arnold[1], Zachary Greenspan[1], Mark Phillips[2], Laurence Mueller[3], Michael R. Rose[3] [1]University of California, Irvine, [2]Department of Integrative Biology, Oregon State University, [3]Ecology & Evolutionary, University of California, Irvine

The evolution and subsequent differentiation of complex traits has been proven to be evolutionarily complicated and genetically pleiotropic. To understand these traits further, we have tracked the genomic evolutionary trajectories of Drosophila melanogaster undergoing selective pressures from well characterized regimes and have begun unpacking the patterns of variation associated with their adaptations. This concentrated effort aims to understand broad genetic architecture in the context of whether patterns of adaptation are heterogenous or homogenous (e.g., are the "routes" of adaptation largely conserved and end points convergent), and what role transposable elements and other genetic structural variants might play in this adaptation process.

Additionally, this work hopes to clarify the role of genetic variation in adaptation using large, outbred, laboratory-domesticated populations and see whether the primary force of evolution follows the general pattern of beneficial alleles sweeping to fixation. Or instead, if evolution generally relies on balancing selection manipulating long standing functional genetic variation. This trajectory project has captured the changes in genetic adaptation by collecting genomic samples at an initial, two midpoint, and terminal timepoints to pinpoint stages of evolution. We have demonstrated phenotypic convergence through life-history characteristics (larval development & age-specific mortality) and have demonstrated the wide-spread shifts in allele frequencies under intense selection.

380S **Exploring the biophysical mechanisms and phenotypic diversity of primordial germ cell specification in *Drosophila*** Chandrashekar Kuyyamudi[1], Emily Rivard[2], Suhrid Ghosh[3], Cassandra Extavour[1] [1]OEB and MCB, Harvard Univeristy, [2]MCB, Harvard Univeristy, [3]Harvard Univeristy

Drosophila primordial germ cell specification is mediated by the inheritance of germ plasm, which contains maternally-derived proteins and mRNAs and is localized at the posterior tip of the embryo. During early embryogenesis, a few posterior nuclei are "immersed" in this germ plasm and bud out to form pole buds that eventually form the PGCs. In *Drosophila melanogaster*, the quantity of germ plasm inherited by the PGCs affects their survival rate, specifically their ability to successfully migrate to the embryonic gonad. We hypothesize that the tight localization of germ plasm at the posterior that is necessary for PGC specification is due to Oskar-mediated formation of large ribonucleoprotein clusters known as germ granules. Our hypothesis suggests that the level of clustering of the constituents affects localization, which in turn determines the quantity of germ plasm components inherited by the PGCs and their ability to successfully migrate to the gonad. Here, we aim to leverage the naturally occurring variability in germ granule size and PGC number that has evolved across *Drosophila* species to test our hypothesis. We quantified the number of PGCs initially formed and surviving to reach the embryonic gonad in several *Drosophila* species spanning the subgenera *Sophophora* and *Drosophila*. Additionally, we are conducting in silico experiments to investigate the effects of embryonic curvature and Oskar's affinity for itself and other germ plasm molecules on the granularity and spatial localization of the germ plasm. Through investigation of the biophysical properties of germ plasm molecules in their specific cellular and tissue-level contexts, we aim to provide insight into the mechanisms underlying germ cell specification and the source of the phenotypic diversity observed in this process across species.

381S **Genomic features and evolutionary formation of long-range chromatin loops across five *Drosophila* species** Aiswaryaa Prabaharan, Jaquelyn Hester, Nicole Torosin, Weihuan Cao, Christopher Ellison Rutgers, The State University of New Jersey

Chromosomes are organized in a hierarchical manner within the 3D nucleus, from chromosome territories to nuclear compartments, topologically associating domains (TADs), and individual chromatin loops. These aspects of the 3D genome have been shown to play an important role in transcription regulation and other epigenetic phenomena. One particularly important component of 3D genome structure are chromatin loops, which are distant regions of chromatin that interact with each other in 3D space. Chromatin loops often involve interactions between promoters and enhancers, which suggests that loops can activate gene expression by grouping regulatory elements together despite their being far apart on the linear chromosome. Although many chromatin loops form within TADs, others span multiple TADs, connecting chromatin megabases apart. It is unclear why these long-range loops form and how they differ from intra-TAD loops. Here, we identify and manually curate over 40 long-range chromatin loops more than one megabase in size by analyzing DNase Hi-C data from *Drosophila melanogaster* embryos. Long-range loop anchors tend to be located in developmentally silenced chromatin and near genes with either brain or testes-biased expression patterns. These loop anchors also connect gene paralogs more often than expected by chance and are enriched for binding sites of a specific class of insulator proteins, but not polycomb proteins. We were able to reconstruct the evolutionary formation of long-range loops via chromosomal rearrangements by analyzing the conservation of long-range loops and gene synteny between *D. melanogaster*, *D. simulans*, *D. erecta*, *D. yakuba*, and *D. ananassae*. Our results provide novel insight into the biological function of these enigmatic genome features and allow us to propose a model of their formation from islands of high occupancy insulator target sites.

382S **A Novel and Sustainable Approach for the Detection of Various Phytonutrients in an Alternate Insect's Protein by Greener Method.** DIVYA SINGH SURESH GYAN VIHAR UNIVERSITY, JAIPUR

One of the most pressing challenges is the growing human population, as there is a great demand for food and nourishment. To combat the problem of food scarcity and nutritional deficits, alternative diet, the insect-based protein is less popular among consumers. In this present work, processed isolated insect's (*Drosophila*) protein powder is solubilized in the mixture of bio based aqueous orange peels extract at a pH of 7.6 and volatile organic solvent (hexane) and is tested for presence of various phytonutrients mainly proteins including amino acids, alkaloids, polyphenols, flavonoids, glucose, tannins and terpenoids *via* greener solvent. The present work is mainly conducted in 4 steps, namely, isolation of *Drosophila* protein, preparation of orange peel extract, detection of various phytonutrients and characterization by FT-IR Spectroscopy. This present research work intends to investigate the action of these phytonutrients in abiotic stress responses by *Drosophila* in future.

383S **Genetic architecture underlying the courtship song divergence between *Drosophila teissieri* and *D. santomea*** Helena Gifford[1], Shen Lin[2], Yun Ding[1] [1]Biology, University of Pennsylvania, [2]University of Pennsylvania

In many *Drosophila* species, males court potential mates by producing courtship songs with their wings. These courtship songs vary between species and play a major role in species recognition and reproductive isolation, providing an excellent model for investigating how species differences in behavioral traits evolve. *D. teissieri* males produce a song consisting of "pulse song" and "sine song". *D. santomea* males produce a song consisting of "clack song" and "pulse song". Intriguingly, hybrids of these two species produce new "chimeric" song types, consisting of clacks followed by a "pulse tail", and pulses interspersed with "sine intervals". To investigate the genetic architecture underlying the evolution of these traits, we leveraged these two hybridizable species and performed a quantitative trait locus (QTL) mapping. We generated a population of 570 *D. santomea* backcrossed individuals, scored the "pulse tail" and "sine interval" characters of their courtship song, and employed multiplexed shotgun genotyping (MSG) to genotype the population. Despite the constraints on mapping resolution imposed by genomic inversions between the two parental species, our initial analyses revealed a highly polygenic genetic basis for both song traits and pervasive epistasis. Specifically, the introgression of a single chromosome from *D. teissieri* into *D. santomea* produced no phenotypic effect on its own, but when combined with the introgression of another chromosome, it resulted in major phenotypic effects. Our ongoing analyses aim to refine the QTL analyses. In summary, this study highlights a polygenic and non-additive nature of the genetic basis underlying an important reproductive trait.

384S **Developmental patterns of transposable element expression in the *Drosophila* embryo at single-cell resolution** Katelyn Boese, Cedric Feschotte, Andrew G. Clark Molecular Biology and Genetics, Cornell University

Although transposable elements (TEs) are often characterized as a deleterious, mutagenic force in the genome, they can also be an important source of genetic novelty for essential processes, such as development. Though many TE-derived genes have established biological roles, there is mounting evidence that proteins contributed by active TEs could be functionally important as well. However, the prevalence of this phenomenon is poorly understood, particularly in invertebrates. To begin assessing the potential role of TEs in invertebrate development, we characterized patterns of TE expression in the *Drosophila melanogaster* embryo using publicly available RNA-seq data. By reanalyzing developmental time-course bulk RNA-seq data generated by the modENCODE consortium, we found that many TE families show broad patterns of expression across all stages, suggesting limited temporal specificity. However, analysis of single-cell RNA-seq datasets revealed several elements with tissue specificity, consistent with early reports of TE expression in the embryo. During gastrulation, LTR elements *412*, *flea*, and *Stalker2* and the DNA element *Hobo* all show expression in various lineages of the mesoderm. In later stages of development (st12-16), the LTR element Dm297 shows strong expression in hemocytes, and this result is also supported by analysis of a bulk RNA-seq dataset of FACS-sorted hemocytes. Thus, we have identified several candidate elements whose somatic tissue specificity, as opposed to selfish germline expression, may suggest acquired developmental function. To determine if these patterns are robust and reproducible in populations with different insertion landscapes, we are currently validating the results in multiple strains by RNA in situ with hybridization chain reaction (HCR). Additionally, we are generating RNAi knockdown lines targeting these elements to test their functional impact.

385S **Investigating the Genetic Architecture of Mutation Rate Variability in Drosophila Populations** Pengyao Jiang[1], Arin Shaw[2,2] [1]Mechanisms of Evolution, Arizona State University, [2]Arizona State University

Naturally occurring mutator alleles, which are the genetic variants that influence mutation rates, remain largely unexplored in natural populations despite their critical role in shaping evolutionary processes. While mutator strains from genetic screens have been extensively studied, little is known about such alleles in nature. Recent studies suggest the existence of naturally occurring genetic modifiers of mutation rates in mouse cohorts, however, their impact remains unclear in other multicellular organisms. In this study, we aim to uncover naturally occurring mutator alleles in Drosophila populations using the Drosophila Synthetic Population Resource (DSPR) recombinant inbred lines (RILs). These lines, derived through initial round-robin mating followed by 50 generations of inbreeding, provide a controlled environment to study de novo mutations. We are processing 705 RILs, each expected to accumulate approximately 519 fixed mutations over 10 years of propagation, with an estimated mutation rate of 1.99 mutations per genome per generation under neutral assumptions. The number of mutations to be discovered in our study is magnitudes higher than current mutation accumulation studies in fly. These genomes will be sequenced using Illumina NovaSeq at 20–30x coverage; we aim to identify novel de novo mutations that arose during laboratory maintenance. We will use quantitative trait loci (QTL) mapping to identify loci influencing mutation rate variation. Together, this work will enhance our understanding of the genetic architecture of mutation rate variability in Drosophila, offering the entire fly community valuable insights into naturally occurring mutator alleles and their potential role in evolution.

386T **Intake of Indigestible Fiber-Rich Diet Induces Mechanical Stress and Modulates *Drosophila melanogaster* Gut Metabolic Homeostasis and Immunity** Abeer Qush, Hadi M. Yassine, Asad Zeidan, LAYLA KAMAREDDINE Qatar University

The intestinal epithelium of mammals consists of several cell types including stem cells (SCs), enterocytes (ECs), enteroendocrine (EEs) cells, goblet cells, and Paneth cells. Among these cells, active and dormant populations of stem cells take part in maintaining the intestinal epithelium, as relevant SCs differentiate into ECs and EEs. While ECs have been shown to be chiefly involved in nutrient absorption, the role of EEs has been mainly attributed to its involvement in nutrient sensing and subsequent secretion of peptide hormones to regulate satiety, peristalsis, and metabolism. As such, EEs, and through peptide hormones, are considered the paramount coordinators of local and systemic responses within the intestine. In addition to nutrient uptake, several external signals including infection, cytokines, and chemicals have been reported to drive intestinal SCs proliferation and differentiation into ECs or EEs. Interestingly, recent evidence from *Drosophila melanogaster* presents mechanical stress as a new signal that contribute to SCs proliferation and differentiation into EEs via the stretch activated Piezo ion channel. This emerging body of evidence opens up for a plausible role of mechanical stress in regulating peptide hormone expression, food digestion, nutrient absorption, gastric emptying, and suggests a possible contribution of mechanical stress in maintaining the overall immune and metabolic homeostatic balance in the gut. As such, we propose herein to study the effect of diet-induced mechanical stress on gut peptide hormone regulation and maintenance of gut homeostatic balance using the *Drosophila melanogaster* model organism. Employing *Drosophila* as the model organism of choice is ascribed to several factors including the cell and organ and immune signaling homology between the fruit fly and mammals, as well as to its ease of rearing and genetic manipulation. Our findings revealed that inducing mechanical stress in the fly gut by means of feeding on non-digestible fiber rich diet elevates the expression of antimicrobial peptide genes, causes a significant alteration in the transcript levels of peptide hormone involved in maintaining metabolic homeostasis, disrupts a number of metabolic parameters including systemic glucose and triglyceride levels, lipid storage levels, and body weight. The results of this study unravel the effect of food-induced mechanical stress on maintaining metabolic and immune homeostasis in the gut, a finding of which could open up for the use of mechanical stress-based therapies to rectify disputed intestinal metabolic and immune homeostatic balance arising from several health conditions in the gut.

387T **A peptide fragment homologous to mammalian C5a derived from the septate junction component Mcr acts as chemoattractant for macrophage recruitment to epithelial wounds** Alessandro Scopelliti[1], Luigi Zechini[1], Henry Todd[1], Thibaut Sanchez[1], Daniel R Tudor[1], Jennie S Campbell[1], Stephen J Jenkins[1], Christopher D Lucas[1], Andrew J Davidson[1], Jean van den Elsen[2], Linus Schumacher[1], Will Wood[1] [1]University of Edinburgh, [2]University of Bath

Aseptic laser-induced epithelial wound in the pupal wing induces a rapid and robust recruitment of circulating macrophages to the injury site, driven by chemotactic signals released from the damaged tissue. However, the nature of the chemoattractant signals generated by damaged tissues remains poorly understood.

Macroglobulin complement-related (Mcr) is a core component of the septate junction, a membrane multiprotein complex that provides paracellular barrier to epithelia.

We show that epithelial Mcr expression is essential for an effective macrophage recruitment to the injury site and this role is independent to septate junction functionality.

Our findings suggest that an extracellular domain of Mcr, homolog to mammalian complement component C5a, plays a crucial role in generating the chemotactic signal driving the inflammatory recruitment of immune cells to injury sites *in vivo*. Our model predicts that tissue damage triggers the proteolytic cleavage of Mcr, resulting in the release of an active peptide that acts as chemoattractant for the recruitment of macrophages. Overall, our work uncovers a previously unappreciated biological function of Mcr that mirrors the chemotactic role of the complement component C5 fragment C5a in mammals.

388T **Ecdysone and Juvenile Hormone regulate intertwined developmental and innate immune processes** Scott A Keith, Vanika Gupta, Brian P Lazzaro Cornell University

20-hydroxyecdysone (20E) and juvenile hormone (JH) have reciprocal effects on immunity, with 20E potentiating and JH suppressing innate immune responses, yet little is known about the molecular bases of these effects. We are investigating the genetic regulatory programs through which these hormones control development, reproduction, and immunity, and how this regulation shapes infection outcome.

JH mediates a physiological tradeoff between reproduction and immunity, as activation of JH signaling in mated females suppresses infection resistance. Using conditional RNAi we discovered that the JH nuclear receptor gce is required in the fat body during metamorphosis for the immune suppressive effects of mating-induced JH signaling in the adult. However, gce in the adult fat body is dispensable for mating-induced, JH-mediated immune suppression. In contrast, we found that JH activated by mating requires both of its paralogous receptors, gce and Met, to suppress immune activation upon infection. These findings suggest that JH signals specifically through gce in the developing fat body to sensitize females to the immune suppressive effects of mating, which require JH signaling through both receptors in adult tissues.

20E activates immunity in developmental and infection contexts but little is known about the transcriptional mechanisms of this activation. We hypothesize that 20E-EcR signaling controls a hierarchical gene regulatory network (GRN) that sustains immune activation in the fat body. To test this hypothesis, we are defining the 20E-regulated GRN using fat body tissue explants treated with exogenous hormone. We found that 20E treatment of ex vivo adult fat bodies induces a transcriptional response characterized by activation of canonical EcR target genes and differential expression of immune genes, including antimicrobial peptides previously suggested to be controlled by 20E during larval-pupal development. Treatment with varied 20E concentrations dramatically altered the magnitude and direction of immune gene expression, suggesting that hormone titer variation could substantially restructure the infection-responsive 20E GRN in the fat body. We are currently using an integrated approach of RNA-seq and CUT&RUN analysis to determine which differentially expressed genes represent direct versus indirect EcR targets. This work aims to better understand hormone-mediated regulation of the physiological balance of development, reproduction, and immunity in the context of host-microbe interactions.

389T **Examining the role of host glycosylation in commensal-host specificity** Andrea M Darby[1,2], Kevin Aumiller[1,2], Haolong Zhu[1,2], Rejeanne Juste[1], Will B. Ludington[1,2] [1]Embryology, Carnegie Science, [2]Johns Hopkins University

The intestinal tract is lined by a mucosal layer rich in glycoproteins that not only protects gut epithelia, but also provides glycosylated ligands for commensal or pathogenic bacteria to bind and consume. Host species across a diversity of taxa show incredible specificity with the gut bacterial communities they associate with, which suggests a role for host genetics in determining microbial communities. Despite our advanced understanding of the role that glycosylation has on the adhesion of bacteria in the gut, we have limited knowledge on the specific factors that determine the glycans produced to selectively bind bacteria.

Recent work from our lab reveals that ligands produced by *Drosophila melanogaster* can recruit bacterial species of interest like *Lactobacillus plantarum* to spatially defined niches in the foregut. *L. plantarum* is a common commensal bacterium found in animals ranging from *D. melanogaster* to humans. We discovered that strains of this species that co-evolved with *Drosophila* express serine rich repeat adhesion proteins that help them bind to the foregut. We hypothesize there are host genetic factors that selectively regulate the production of glycans in specific regions in the gut to promote selective adhesion of *L. plantarum*, which could support metabolic function and overall health of the host.

To test this hypothesis, we are conducting a genetic screen using the Gal4-UAS system to knockdown expression of identified candidate genes that are (i) specifically expressed in the foregut, (ii) secreted), and (iii) highly expressed. We measure these effects on L. plantarum's ability to colonize. Our initial results show that several RNAi knockdowns of genes responsible for N-linked and O-linked glycosylation in the foregut significantly reduce *L. plantarum* colonization. These mutants will be further investigated using additional genetics approaches and phenotyping by bellymount imaging to visualize the localization and adhesion of *L. plantarum* in the foregut.

390T **One Hot Paradox: Investigating the Antiviral Effects of HSP90 Inhibition in *Drosophila melanogaster*: Insights into Heat Shock Response, HSF, and Viral Replication Dynamics** Ella Buhlke[1], Blase Rokusek[2], Darby Carlson[3], Sunayn Cheku[3], Kimberly A Carlson[3] [1]Biology, University of Nebraska at Kearney, [2]University of Nebraska Medical Center, [3]University of Nebraska at Kearney

The heat shock response (HSR) was discovered and has been extensively studied in *Drosophila melanogaster.* This organism should serve as a good model in which to study the interaction of viral infection and the HSR. Yet very little research involving this interaction has been conducted in *D. melanogaster.* The purpose of the present investigation was to validate the antiviral effect of pharmacological inhibition of HSP90 *in vivo* in a *D. melanogaster* model, which has never before been shown. Further, we sought to explore other aspects of the relationship between the HSR, heat shock factor (HSF), and viral infection. Specifically, we explored the affect that pharmacological HSP90 inhibition with 17-allylamino geldanamycin (17-AAG) and 17-desmethoxy-17-N,N-dimethylaminoethylamino-geldanamycin (17-DMAG) on estimates of viral load at 24 and 72 hours after persistently infected stocks of *D. melanogaster* received treatment. We are also examining the ratio of the negative, replicative strand of the virus to the positive strand to understand replicative efficiency. Also tested was the treatment with Direct Targeted HSF-1 InhiBitor (DTHIB) on persistently infected stocks with heat shock treatment at 36.5°C for one hour to examine the relationship between the two. We also report a trend whereby a line of *D. melanogaster* carrying a mutant *HSF* tended to have higher estimates of viral load relative to genetic controls with wild type *HSF.* Finally, we found that DmNV infection leads to significant elevation of *HSP83* (HSP90; $P = 0.029$) and *DNAJ-1* (HSP40; $P = 0.001$), 24 hours after infection. Our data suggest a prominent role for the HSR and associated inducible HSPs during DmNV infection *in vivo.*

391T ***NinjurinA (NijA)* is necessary for survival following Invertebrate Iridescent Virus 6 (IIV6) infection of adult *Drosophila*** Molly Murphy, Neal Silverman Medicine, University of Massachusetts Chan Medical School

Invertebrate Iridescent Virus 6 (IIV6) is a large DNA virus encoding approximately 200 genes that infects a wide range of insects. While not a natural host, *Drosophila* can be experimentally infected by IIV6 which causes a slowly lethal infection and displays strong suppression of host defense responses. This suppression includes inhibition of the antibacterial Toll and Imd pathways as well as the antiviral RNAi pathway. Instead of relying on one of these classical innate immune pathways, *Drosophila* instead induce a cytokine-triggered JAK/STAT-mediated response that drives the expression of *Turandot* (*Tot*) genes[1]. While Tot proteins have recently been implicated in anti-tumor responses[2] as well as protection from self-damage by AMPs[3], their role in antiviral defense remains unclear. In our efforts to further characterize the *Drosophila* response to IIV6 we found *NinjurinA (NijA)*, encoding a protein involved in cell membrane rupture, was upregulated upon IIV6 infection of *Drosophila* DL1 and S2R cells. Null mutant $NijA^{D3}$ flies were significantly more susceptible to IIV6 infection; however, *Tot* induction was unaffected when measured in whole flies. These findings present interesting questions regarding the role of *NijA* and plasma membrane rupture in anti-viral host defense, as well as on the molecular mechanisms of virus-triggered *Tot* induction.

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392T **Microbiome-Metabolite Composition of Natural Diet in Drosophila's Metabolic Fate** oluwatobi E fijabi, laura K Reed Biological Sciences, University of Alabama

Host-microbe interactions play a crucial role in modulating metabolic health. Given the extensive diversity of microbial populations found in rotting fruits, how these interact with the fruit's chemical composition determines the molecular profiles of the rots, which influences the host's biology. To enhance our understanding of the human metabolic syndrome, we have characterized a tripartite symbiosis involving fruit diets, their microbial communities, and *Drosophila*. We hypothesize that altering the microbial populations in diets will provide insights into metabolic dysbiosis and that *Wolbachia* may improve the host›s fitness under nutritional stress. To investigate this, organic peach and strawberry fermented diets were heat sterilized and/or treated with antibiotics, then fed to L1 axenic larvae from DGRP lines until they reached L3.

Global metabolomic analysis of these diets revealed that trehalose sugars and amino acids were more abundant in the strawberry diet, while 16S sequencing detected elevated levels of *Gluconobacter oxydan* in the peach diet. The antibiotics had minimal impact on the overall metabolite profile of the diets, primarily showing a decrease in carbohydrate metabolite derivatives, while autoclaving resulted in the degradation of phenols, flavonoids, and amino acids. Additionally, autoclaving increased alpha-tocopherol and Maillard reaction products. , such as 5-methyl-2-furancarboxyladehyde, which is a marker for Type 2 Diabetes and cardiovascular disease. Larvae fed autoclaved diets exhibited symptoms of dysregulation in pathways related to glycerol-lipids, biogenic amines, glutathione, vitamin B, and phenylalanine/tyrosine. Larvae that received antibiotic-treated diets showed increased cell wall degradation metabolites like diaminopimelic acid and cellobiose, along with increased cofactors, bioactive compounds, and saturated fatty acids. 16S data indicated that *Drosophila* that were fed treated diets experienced reduced gut diversity, including a reduction in beneficial taxa. Diversity loss was specific to the fruit and treatment (autoclaving or antibiotics). Furthermore, beneficial bacteria were significantly diminished in those fed autoclaved diets. *Wolbachia* also elevated levels of some metabolites compared to the *Wolbachia*-free flies on the autoclaved diet. Flies infected with *Wolbachia* exhibited a pronounced increase in uncharacterized bacteria taxa, while simultaneously depleting *Eubacterium, Bacillus, Clostridia,* and *Firmicutes*. These findings are pivotal for advancing our understanding of insect ecology and demonstrate how dietary metabolites and the microbiome fundamentally influence metabolic outcomes in humans.

393T **The inducible expression of *Diedel* by multiple immune signaling pathways** Bao Ho[1], Peter Nagy[2], Nicolas Buchon[2], Neal Silverman[3] [1]Medicine, UMass Chan Medical School, [2]Cornell University, [3]UMass Chan Medical School

The Imd, Toll and JAK/STAT pathways control inducible innate immune defenses in *Drosophila melanogaster*. The activation cascades from each of these three pathways are well-studied, but it is not clear how they might intersect or collaborate with one another to control immunity and immune-inducible gene expression. We hypothesized that these pathways do intersect, which will become evident in a set of genes that need 2 or all 3 to become activated following a particular immune challenge. We devised an experiment in which mutants deficient in each of these three pathways were challenged with several immune stimuli and then analyzed the response with a transcriptome-wide analysis. Among the genes induced by multiple stimuli and involving multiple pathways, *Diedel* stands out. In wild-type flies, Diedel is transcriptionally induced by infection with Gram-negative *Ecc15* (*Pectobacterium carotovorum*). Although it is the response to Gram-negative bacteria primarily requires activation of the Imd pathway, *Diedel* induction by *Ecc15* required all three pathways (Imd, Toll and JAK/STAT). Further investigation revealed the DAP-type peptidoglycan component from the Gram-negative bacteria cell wall is sufficient to trigger induction of *Diedel* expression in the wild-type background. Yet, the transcriptional induction of *Diedel* expression required the upstream activating components of Toll pathway: *Spätzle*, its cleaving enzyme *SPE*, as well as the peptidoglycan sensor *PGRP-SD*, and to a lesser extent the serine proteases *psh* and *modSP*. In this study we investigate the mechanisms of *Diedel* regulation by these three pathways.

394T **Drosophila Mcr is a functional homologue of mammalian complement C5a and operates as a wound induced chemotactic signal to drive inflammatory cell recruitment to sites of sterile tissue damage** Luigi Zechini[1], Alessandro Scopelliti[1], Henry Todd[1], Thibaut Sanchez[1], Daniel Tudor[1], Jennie Campbell[1], Stephen Jenkins[1], Christopher Lucas[1], Andrew Davidson[2], Jean Van Den Elsen[3], Linus Schumacher[1], Will Wood[1] [1]University of Edinburgh, [2]University of Glasgow, [3]University of Bath

Sterile tissue injury triggers an acute inflammatory response, with innate immune cells quickly migrating to the injury site in response to chemotactic signals released by the wound. The identity and mechanisms of the earliest signals that drive immune cell recruitment within the complex multicellular environment of a living organism remain poorly understood.

In this study, we use Drosophila genetics and live imaging, combined with mathematical modeling, to identify the fly complement ortholog, Macroglobulin complement-related (Mcr), as an essential component of early wound-induced chemotactic signal essential for recruiting immune cells to injury sites in vivo.

We demonstrate that epithelial-specific knockdown of Mcr significantly reduces macrophage recruitment to wounds. Through the combination of predictive mathematical modeling and genetic manipulation, we elucidate how Mcr shapes macrophage migration dynamics. Our work supports a two-signal model, in which Mcr works alongside hydrogen peroxide to drive a rapid and efficient immune response to tissue damage, revealing a novel role for Mcr that mirrors the chemotactic function of the mammalian complement component C5a.

395T **Young *Drosophila* suggests a downregulation of the IMD pathway following exposure to exercise and infection** Tolulope R Kolapo[1], Laura Reed[2] [1]Biological Sciences, The University of Alabama, [2]Biological sciences, The University of Alabama

Exercise is a beneficial and non-invasive remedy to obesity and other metabolic disorders. With the multi-faceted effects of exercise on various biological processes and systems, there is a need to understand the molecular mechanism through which exercise impacts the system both in an infected state and in healthy individuals. Innate immunity involves increased antimicrobial activity through the expression of antimicrobial peptide genes (AMPs) to fight pathogens and the upregulation of the pathways of inflammation. *Diptericin, and Attacin* are AMPs downstream of the Immune deficiency (IMD) pathway that are triggered by gram negative bacteria invasion. Also, the *Upd3* and *cnc* genes are critical regulators of the antioxidative and anti-inflammatory pathways.

Drosophila is a model organism that has been used to either study innate immunity or exercise. However, there are no known studies that have considered these two factors simultaneously to further identify the effect of exercise on molecular pathways in immune-activated individuals. Our study focused on modeling the impact of prior exercise exposure on response to bacterial infection in young *Drosophila*.

We determined the antimicrobial activity levels in infected-exercised and uninfected-exercised flies. For three Drosophila Genetic Reference Panel (DGRP) lines, five-day-old flies were exercised for two weeks then infected with gram-negative bacteria (*Escherichia coli*), incubated for six hours, then frozen. We considered the effect of exercise on three pathways, IMD, JAK/STAT, and NRF2/Keap1, by quantifying the expression of *Diptericin* (*Dpt*), *Attacin* (*Atta*), *Unpaired Protein 3* (*Upd3*), and *cap and collar* (*cnc*) genes respectively.

Results from the study reveal a significant decrease in the expression of *Diptericin* in young, trained females' post-infection across two genotypes. We also see decreased *Diptericin* in exercised flies before infection which is consistent across three genotypes. We establish that regardless of genotype and sex, exercise suppresses the Immune Deficiency pathway. However, there is no significant effect of exercise and infection on the expression of *Upd3* and *cnc*, genes that are critical regulators of the JAK/STAT and NRF2/Keap1 pathways. This study suggests that prior exposure to exercise impacts how a biological system fights infection. Genetic variation and sex further determine the extent to which exercise impacts response to infection.

396T **The microbiota contributes to seasonal adaptation of *Drosophila melanogaster*** Dean Peterson[1], John Chaston[2], Peyton Jackson[2], Hyrum Pech[2], Kelly Moon[2] [1]Life Sciences, Brigham Young University, [2]Brigham Young University

The fruit fly *Drosophila melanogaster* is a model for understanding the rapid responses of organisms to their changing environments, or adaptive tracking. Our previous investigations of adaptive tracking in flies responding to seasonally varying pressures showed that the microbiota can be an agent of host genetic selection. However, this previous work only investigated fly responses over 6.5 weeks of seasonal selection. To better understand how the microbiota influences adaptive tracking in *D. melanogaster* we subjected flies in outdoor mesocosms to 18 weeks of selection across a full summer-to-fall season in NH, USA, while inoculating their diets with microorganisms that lead to divergent host phenotypes. Fly population sizes, which estimated the bacterial influence on fly fitness, differed between bacterial treatments. Bacterial strains that maximized fly fitness in peak of summer led to the poorest fitness outcomes in the fall, and vice versa. We also compared fly life history traits between the fly treatments in their offspring, reared in the laboratory as bacteria-free flies, four times during the selection period. Differences in the bacteria-free fly phenotypes of the adapting flies showed that inoculating the flies with bacteria led to genetic differences between the flies, and that certain bacterial functions relieved seasonal selective pressure on the flies. Together, these findings provide insight into how exposure to changing environments and microbial communities can influence rapid adaptation in a model animal host.

397T **Re-Characterizing the Role of Thor in *Drosophila melanogaster* Immune Dynamics** Kate L Browning[1], Jailyn Loor[2], Brian P Lazzaro[1] [1]Entomology, Cornell University, [2]Cornell University

Thor encodes eIF4E-BP, which inhibits cap-dependent mRNA translation under oxidative stress, starvation, and bacterial infection in *Drosophila*. Previous studies had indicated that *Thor* mutant flies are highly sensitive to Gram-positive (G+) and Gram-negative (G-) bacterial infections as adults. In larvae, it was reported that upregulation of *Thor* through the GCN2-ATF4 pathway biases translation towards production of antimicrobial peptides in the gut during enteric infection. We initially aimed to test Thor-dependent regulation of translation in the adult fat body during systemic bacterial infection. However, while performing genetic background isogenization, we discovered that the infection-sensitivity phenotype could be separated from the *Thor* mutation through a single recombination event. After backcrossing *Thor* mutant flies to wild type Canton S for 10 generations, we observed no difference between the *Thor* mutant and wildtype flies in susceptibility to infection with intermediate-virulence bacterial pathogens *P. rettgeri* (G-), *S. marcescens* 2698B (G-), and *L. lactis* (G+). We then generated 54 lines carrying a unique second chromosome from recombining the original $Thor^2$ mutant strain and wild type Canton S, and tested each of these for susceptibility to *P. rettgeri* infection. We find that infection sensitivity is independent of the *Thor* mutation and presumably results from a mutation in the background of chromosome 2. We have sequenced the parent and recombinant flies and are mapping the recombination breakpoints to identify candidate genes that may be responsible for observed sensitivity to infection. Interestingly, our backcrossed *Thor* mutant flies remained susceptible to *E. faecalis* (G+) infection even after backcrossing to Canton S. We are thus also investigating the role of Thor in response to systemic *E. faecalis* infection, and why the response to *E. faecalis* is distinct from the response to other bacterial pathogens.

398T **Surviving the STING of Infection Through TOR** Carly Lam[1], Janet Phang[2], Katarina Akhmetova[3], Igor Chesnokov[3], Michele Shirasu-Hiza[1] [1]Columbia University Medical Center, [2]Columbia University, [3]University of Alabama at Birmingham

The Stimulator of Interferon Genes (STING) pathway is a highly conserved innate immune pathway that protects organisms from invading pathogens through induction of antimicrobial molecules such as interferon and anti-inflammatory cytokines. Most of the research on STING has focused on its critical role in immunity. Recently, STING has also been implicated in metabolic regulation, most recently interacting with the Target of Rapamycin (TOR) pathway, a nutrient-sensing pathway that regulates cell growth, cell proliferation, cell survival, protein synthesis, and autophagy.

Using *Drosophila melanogaster* as an animal model, I infected *Sting* mutants with the opportunistic pathogen *Burkholderia cepacia* and observed a surprising phenotype. *Sting* mutants, even though they lack a critical immunity regulator, survived this infection longer (not shorter) than isogenic wild-type controls while having the same bacterial load. These results suggest that STING may play roles in both infection resistance (innate immunity, as established by others) and infection tolerance (ability to withstand the pathogenic effects of infection). My lab had previously observed this same phenotype of increased infection tolerance with TOR complex mutants. TOR kinase can be found in two distinct complexes: TOR Complex 1 (TORC1), which is inhibited by rapamycin, depends on Raptor, and is implicated in adaptive immunity and aging, and TOR complex 2 (TORC2), which is much less well understood than TORC1. My lab had found that, with depletion of dietary amino acids, genetic mutants lacking TORC2-specific components rictor or Sin1 survived B. cepacia infection longer than isogenic controls. Using genetic and molecular approaches, I am currently testing the hypothesis that TORC2 and STING act in the same pathway to regulate infection tolerance. These results will reveal possible links between resistance and tolerance pathways and also provide insight into much-needed therapeutic targets for increasing survival of infection independent of pathogen load.

399T **The influence of the microbiome on *Drosophila* flight performance** Maria Lovallo, Eliana Garza, Nichols Robert, Emily Davenport, Claire Thomas Biology, The Pennsylvania State University

Microbiomes and their hosts have co-evolved and have mutual effects on their development and physiology. *Drosophila* species are colonized by a variety of aerobic and anaerobic microbes, which have been linked to a variety of physiological outcomes in the host including immunity, longevity, and fecundity phenotypes. However, whether the presence of or composition of the microbiome affects flight performance - a phenotype of high fitness consequence - is not well understood. Moreover, the physiological mechanisms, including specific microbial metabolites involved in regulating this process remain uncharacterized.

To address these open questions, we are using multiple physiological assays to determine the impacts of the microbiome on flight performance in axenic and gnotobiotic *Drosophila melanogaster* both with and without the endosymbiont *Wolbachia pipientis*, which is known to impact both microbiome composition and host physiology. We hypothesize that microbiome has the potential to alter flight performance and are testing how the presence and composition of the microbiome influences flight endurance and acute flight recovery in a drop test. We hypothesize that axenic organisms may exhibit different performance in these assays due to their lower body weights or variation in microbiome-derived nutrient availability. These physiological assays are paired with metabolomic screening *via* LC-MS of thoracic flight muscle extracts to identify differences in key metabolite levels related to cellular energy availability between flies of different microbial colonization status.

This study will provide key insights into the specific influences of the microbiome on flight behavior in *Drosophila* and provides a platform to explore the interaction between the host genome and microbiome with direct impacts on evolutionary fitness in the environment.

400T **_Wolbachia_ bacterial symbionts protect _Drosophila_ hosts against fungal pathogens** Jessamyn I Perlmutter, Aylar Atadurdyyeva, Margaret Schedl, Robert Unckless Molecular Biosciences, University of Kansas

Often called “the world’s greatest pandemic”, *Wolbachia* bacteria of arthropods are the most widespread endosymbionts on the planet. They are found in over half of all insect species and exist on every continent except Antarctica. These bacteria are vertically transmitted from mother to offspring via the cytoplasm much like mitochondria. To ensure faithful inheritance of the symbiont, *Wolbachia* rely on a set of cunning host interactions to enhance the fitness of hosts carrying the symbiont. Crucially, one phenotype is the ability to inhibit viral pathogens when an arthropod host contains certain *Wolbachia* strains. Mosquitoes with the *w*Mel *Wolbachia* strain of *Drosophila melanogaster*, for example, exhibit significantly reduced transmission rates of viruses like dengue and chikungunya to new hosts. Global initiatives have thus far achieved great success in using *Wolbachia* in the wild to reduce mosquito-borne transmission of viral diseases to humans. However, very few studies have focused on *Wolbachia*’s interactions with other microbes such as fungi despite the prevalence of fungal pathogens in the wild. Here, we perform a comprehensive panel of fungal infection assays with *Drosophila melanogaster* flies with or without *Wolbachia* to fill this gap. We find that the symbiont protects hosts against a wide array of filamentous and yeast pathogens of agricultural and medical importance by increasing host longevity post-infection. Notably, the presence and strength of the phenotype is dependent on factors including host genotype, sex, and fungal species. Flies co-infected with *Wolbachia* and fungus have lower pathogen titers and exhibit higher egg-laying, indicating that the mechanism is likely host resistance and that the phenotype confers a significant fitness benefit. Further, flies with the symbiont have increased expression of key immune genes post-fungal infection relative to flies without the symbiont, suggesting induction of host immunity may be key to *Wolbachia*’s ability to fight fungal pathogens. This study represents a major advancement in *Wolbachia* research and applications by demonstrating robustly that the famous pathogen-blocking abilities of *w*Mel can now be broadly extended to another major branch of microbial life. These results not only provide new information on how *Wolbachia* have achieved such a high global prevalence, but will also inform pathogen-blocking research and open up new possible applications of *Wolbachia* to fighting fungal pathogens.

401T **The epithelial sheath acts as a barrier to professional phagocytes in response to apoptosis in the ovary** Max C Wertheimer[1], Alexandra Chasse[2,3], Kim McCall[1] [1]Biology, Boston University, [2]Boston University, [3]St. Jude Children’s Research Hospital

In Drosophila, when cells die, they are typically removed by phagocytic hemocytes. The ovary of Drosophila possesses nonprofessional phagocytes in the form of follicle cells that engulf apoptotic cells instead of hemocytes, as they have little to no presence inside the ovary. It is unknown how the ovary bars entry to hemocytes when dying egg chambers release signals during cell death events for engulfment. The signals released typically attract phagocytic cells to break down developing egg chambers and recycle nutrients but while hemocytes have been seen on the periphery, they infrequently enter the ovaries. The epithelial sheath, which lines the surface of ovarioles, may inhibit hemocyte infiltration via septate junctions which control the diffusion of signals across tissues and may physically inhibit hemocytes themselves. Eleven septate junction genes were identified as candidates and were knocked down in the epithelial sheath to determine if they played a role in barring hemocyte entry into the ovariole. Many of the selected genes had adverse effects on fecundity and showed abnormal ovary structure. Two of the gene knockdowns showed a decreased number of midstage egg chambers and increased hemocyte infiltration. These knockdowns also showed an increase in midstage cell death. Ongoing analysis is aimed at determining whether the infiltrating hemocytes cause the death of the egg chambers or the death of egg chambers attracts hemocytes. Discovering the gene(s) responsible for epithelial sheath permeability can be key for understanding the immune response in other immune privileged organs and potentially develop targets for therapy.

402F **Illuminating the non-genetic factors of immune activation** Yu Yang Boston University

Immune activation is a tightly controlled process, as over-activation leads to autoimmunity, while under-activation lets bacteria proliferate unchecked. Even genetically identical *D. melanogaster* infected with the same number of bacteria will die at varying times due to random individual variation and pathogen growth trajectories. The exact interplay between bacteria growth and immune response in individual animals has yet to be observed due to the difficulty of obtaining individual and real-time data. I can estimate bacterial growth by dilution plating a representative sample but losing the nuance of individual immunity over time. However, previous research suggests that variation in the ability of genetically identical flies to survive an infection depends on how rapidly the immune system activates to control the bacterial load early in the infection. By observing and modeling the individual variation in immune response, we can discover new non-genetic targets that control immune activation. I will approach this question using bioluminescent bacteria, allowing me to continuously track infection progress in individual flies in a high throughput manner. The generated bioluminescence signal is closely correlated with bacterial load in the fly. I will use gram-positive and gram-negative bacteria to probe the activation speed and variability of the two major innate immune pathways: Toll and IMD. Concurrently, I will track fly immune response using flies that produce GFP when producing antimicrobial peptides (AMP) such as *diptericin* and *drosomycin*. I will image the Dpt-GFP and Drs-GFP enhancer reporter flies injected with bioluminescent bacteria, tracking how the luminescence signal changes in response to immune activation. Using *D. melanogaster*, I aim to develop new imaging methods to observe host-pathogen interactions, in a model system ideal for studying conserved features of innate immunity in the absence of an adaptive immune response.

403F ***Wolbachia* tissue distributions and effects on components of fitness across divergent *Drosophila* host species**

John Statz[1], Brandon Cooper[2] [1]The University of Montana, [2]Division of Biological Sciences, The University of Montana

Maternally transmitted *Wolbachia* associate with at least half of all insect species. Most *Wolbachia* form facultative host associations and occur at a range of frequencies. The spread and equilibrium frequencies of facultative *Wolbachia* depend on their rates of maternal transmission and effects on host fitness and reproduction. Reproductive effects include male killing, feminization, and cytoplasmic incompatibility (CI). The intensity of *Wolbachia* effects on hosts – and their likelihood of successful transmission – ultimately depends on their abundance and distribution across host tissues. Here, we employ novel whole-mount confocal imaging to quantify *Wolbachia* in the reproductive, digestive, and nervous tissues of five divergent *Drosophila* systems that have been foundational for understanding *Wolbachia*-host interactions. We also report a comprehensive analysis of *Wolbachia* effects on host fecundity and viability for all five systems, in the context of our imaging analyses. Our systems include *w*Mel, first discovered in *D. melanogaster* and now observed across holometabolous insects diverged up to 350 million years (MY), and *w*Ri, first discovered in *D. simulans* and now observed in *Drosophila* hosts diverged up to 50 MY. We observe interspecific variation in patterns of *Wolbachia* tissue distributions that range from specific tissue localization to widespread occurrence across all tissues. Analyses of *w*Ri in *D. simulans* and *w*Ri-like *Wolbachia* (*w*Aur) in divergent *D. auraria* demonstrate that *Wolbachia* diverged only a few thousand years can differ widely in their somatic distributions and abundances. Specifically, *w*Ri occurs at high abundance in the central nervous system, which may underlie its positive effects on *D. simulans* fecundity and viability and its rapid spread through global *D. simulans* populations. We are now evaluating reciprocally introgressed genotypes within host systems to determine the relative contributions of host and *Wolbachia* genomes to this trait variation. We also observe that *w*Mau occurs at excessively high abundances across *D. mauritiana* tissues, corresponding to negative *w*Mau effects on *D. mauritiana* egg-to-adult viability. This implies that dysregulated *Wolbachia* abundances may negatively influence host fitness components. Together, these data contribute to our long-term goal of elucidating the evolutionary-cellular-genetic basis of *Wolbachia* effects on hosts that underlie their spread within and between divergent insect systems.

404F **Characterization of Metabolomic and Behavioral Changes in Young and Old Drosophila Adults Mono Associated with Probiotic Lactiplantibacillus plantarum** Melanie Reinoso[1], Caroline Casiano[2], Josue Rodriguez-Cordero[2], Alfredo Ghezzi[2], Jose Agosto[2], Imilce A Rodriguez-Fernandez[2], Charles Pfeiffer[2] [1]Biology, University of Puerto Rico Rio Piedras, [2]University of Puerto Rico Rio Piedras

The gut microbiota-brain axis is a bidirectional communication between the resident microbes, the gut, and the brain; it plays a pivotal role in aging and age-related diseases. Probiotics have emerged as a promising avenue for interventions targeting age-related diseases by modulating this axis. Of particular interest is how certain probiotics can influence the host metabolome, a mechanism that could further our understanding of bacterial effects on the gut-brain axis. To study this we are using *Drosophila melanogaster* as a genetically amenable model that displays age-related phenotypes and has a simple microbiota that is easy to manipulate. To explore the effects of the commensal bacteria and probiotic *Lactiplantibacillus plantarum* (formerly *Lactobacillus plantarum*) on the metabolome of young and old *Drosophila*. First we aged flies to 5 days (young) or 45 days (old), then we treated them for 5 days with an antibiotic cocktail to generate Antibiotic-Induced Microbiome-Depleted (AIMD) flies. Then, we mono-associate them with *L. plantarum* for 1 day. Flies were flash-frozen and separated by heads (brain) and bodies (gut) and collected separately. From a total of 855 biochemicals, we identified changes in metabolites that are age-, tissue- and treatment-specific. PCA analysis revealed as expected there was a distinct separation between age and body parts. Although the influence of *L. plantarum* supplementation on the metabolome appeared relatively modest, there were significant differences in neurotransmitters, specifically acetylcholine which is increased in the bodies of old flies treated with *L. plantarum*.

Next, we explored the effects of *L. plantarum* on locomotion and sleep in conventional and AIMD-raised flies. Flies were treated for 3 days with either a 5% sucrose solution as a mock control, one of two *L. plantarum* strains (wild-type fly-derived LpWF or cabbage-derived Lp39), or E.coli as a negative control in 5% sucrose. Locomotor and sleep behaviors were assessed using the Trikinetics DAM2 monitoring system. Results indicated that AIMD flies treated with *L. plantarum* exhibited an increase in overall sleep duration and a decrease in sleep latency but the latter is not specific to *L. plantarum* as *E.coli* had the same effect. In terms of locomotion, *L. plantarum* seems to rescue a hyperactive phenotype exhibited by AIMD flies. Ongoing experiments are characterizing if these *L. plantarum* phenotypes are dependent on neurotransmitters such as acetylcholine.

405F **Feeding promotes the environmental transmission of an insect endosymbiont** Dylan Shropshire, Alphaxand Njogu, Helene Hartman, Callum Shutack Department of Biological Sciences, Lehigh University

Wolbachia is the most prevalent endosymbiotic bacterium, inhabiting the cells of over 50% of insect species worldwide, including *Drosophila melanogaster* and its relatives. As it is increasingly deployed across multiple continents to combat mosquito-borne diseases like dengue and Zika, understanding *Wolbachia*'s transmission dynamics has become critically important. While it is primarily transmitted vertically from mother to offspring, growing evidence indicates that *Wolbachia* can also spread horizontally between species. However, the mechanisms and conditions that enable such host switching are still poorly understood. A crucial step in this process likely involves the release of *Wolbachia* from host cells into the environment. We hypothesize that food may act as a conduit for *Wolbachia*'s environmental transmission. I present findings from a series of experiments that support this hypothesis, revealing key dynamics of *Wolbachia*'s environmental transmission and offering new insights into its widespread distribution and potential interactions with new hosts.

406F **Characterization of the Nuclear Localization Signal (NLS) of ORF1 of Nora virus** Belle Turk[1], Amanda J Macke[2], Darby J Carlson[3], Alexis Hobbs[3], Kimberly A Carlson[3] [1]Biology, University of Nebraska at Kearney, [2]University of Nebraska Medical Center, [3]University of Nebraska at Kearney

Nora virus is a picorna-like virus that is endemic in *Drosophila melanogaster* and referred to as *D. melanogaster* Nora virus (DmNV). The genome of DmNV contains four open reading frames (ORFs) known as ORF1, ORF2, ORF3, and ORF4. ORF1, the focus in this study, has a role in RNA interference, RNAi, suppression through inhibition of the RNA induced silencing complex, RISC. This allows Nora virus to remain persistent in its host. Sequence analysis of ORF1 shows not one, but four potential putative bipartite nuclear localization signal (NLS) sites. The NLS 2 and 3 sites overlap and are considered together as one NLS or NLS 2. Knockout mutations for NLS 1, 2, 3, 1 & 2, 1 & 3, 2 & 3, and all three knockout mutations together (1, 2, & 3), were created and cloned into the *pCR-TOPO* vector. DNA sequencing verified that the intended mutations were created. The verified mutants were subcloned into *pEGFPN3* for transfection into S2 cells. The S2 nucleus will be stained with DAPI, and cells visualized using confocal microscopy. This study will let us determine the identity of the ORF1 NLS responsible for translocation of DmNV to the nucleus.

407F **Effect On Microbiome Composition in the *park[25]* Mutant Model of Parkinson's Disease** Paige E. Bonnette[1], Shelby C Olson[1], Samantha M. Chagolla[2], Krista Pearman[3], Haolong Zhu[4,5], William B. Ludington[4,5], Gerald B. Call[1,3] [1]Arizona College of Osteopathic Medicine, Midwestern University, [2]College of Dental Medicine Arizona, Midwestern University, [3]Department of Pharmacology, College of Graduate Studies, Midwestern University, [4]Biology, Johns Hopkins University, [5]Embryology, Carnegie Institution for Science

Parkinson's disease (PD) is characterized primarily by motor and neurological symptoms. Many PD patients experience other symptoms involving gastrointestinal issues including gut dysbiosis and constipation. To study the potential of the gut brain axis (GBA) in PD, a Drosophila melanogaster model of PD (*park[25]*), was utilized. Previous work using axenic embryos inoculated with one of four different bacterial strains showed a large increase in bacterial colonization when compared to control. To further isolate the colonization, flies monoassociated with *Lactobacillus plantarum* were dissected to separate different regions of the gut for homogenization and culturing. The subsequent bacterial colonies were counted and the colony forming units (CFUs)/fly were calculated. The crop was shown to have increased colonization when compared to control. Inoculation of *park[25]* flies with fluorescently labeled *L. plantarum* visualized using confocal microscopy supported this finding. To investigate if loss of *park* in the crop was responsible for this result, crop-specific expression of *park*-RNAi constructs were used. The knockdowns had no significant increase in colonization when compared to their controls, indicating that loss of *park* in the crop is not responsible for the CFU overload. A constipation assay was performed to look at gut motility in *park[25]* and trans-heterozygous *park* flies. Results of this assay indicate that *park[25]* have the same motility as control flies, while transheterozygous flies have decreased motility compared to control flies. Additionally, no flies had leaky guts via the smurf assay. Further investigation of transheterozygous *park* flies did not show the increase in colonization that is present in the *park[25]* fly, both with CFUs and fluorescent visualization. These results indicate the loss of *park* is not responsible for this phenomenon. Therefore, it is likely that the increased colonization observed in the homozygous *park[25]* fly could be evidence of a background mutation in this model.

408F **Exploring *Drosophila melanogaster* as a Model for Peanut Allergy Research: Immune Pathway Responses and Gene Regulation Insights** Carlos Hernandez[1], Adelaide Buhlke[2], Alexis M Hobbs[3], Joseph J Dolence[2], Kimberly A Carlson[2] [1]Biology, University of Nebraska at Kearney, [2]University of Nebraska at Kearney, [3]Biology

The use of *Drosophila melanogaster* for the study of peanut allergies is not common, although it is effective, and budget friendly. *D. melanogaster* is useful for human studies due to similarities between disease-related genes and immune response pathways. The objective of this study was to determine if the immune-regulated genes within the *D. melanogaster* genome were affected by the exposure to peanut. For this study, eight hundred female flies were collected and placed into cages, one hundred per cage. The flies were fed cornmeal-molasses food with water or 5% peanut on top, with water being the control. Every 72 hours, the dead flies were collected, and food was replaced. qRT-PCR was performed at three-day intervals across the lifespan of the flies. These results show a significant down-regulation of *Dorsal* and an up-regulation of *Dif, Cactus,* and *Relish*. This shows that the Toll pathway is potentially involved in allergic reactions, as well as the Immune Deficient (IMD) pathway. The experiment was repeated, and females were collected on days 0, 15, and 30 for Next Generation Sequence (NGS). The results show at day 15 an upregulation of the genes: *Cp18* (Chorion protein 18), which is involved in chorion formation, and *Jon25Bi* (Jonah 25Bi), which enables serine hydrolase activity. Day 30 shows an upregulation of *Npc2e* (Niemann-Pick type C-2e), which is involved in immune signaling via LPS, lipid A, peptidoglycan, and lipoteichoic acid, and the IMD pathway, and a downregulation of the genes: *Jon25Bii* (Jonah 25Bii), which enables serine hydrolase activity, *Gnmt* (glycine N-methyltransferase), which encodes enzyme that catalyzes methylation of glycine to N-methylglycine (sarcosine), and *rib* (ribbon), which encodes a BTB-domain protein required for the development of salivary gland and trachea, also the potential for regulation of PIWI interacting RNAs (piRNAs; i.e., viral infection fighting). *In toto*, this data demonstrates that *D. melanogaster* provokes an immune response to peanut exposure and can potentially be used as a model for peanut allergy.

409F **A Potential Role of Lactiplantibacillus plantarum in Modulating behavior via GABA Signaling in Drosophila melanogaster** Angel Alexander Torres-Roman, Caroline V. Casiano-Rivera, Imilce Rodriguez-Fernandez University of Puerto Rico, Rio Piedras Campus

The gut-brain axis, influenced by gut microbiota such as bacteria, viruses, fungi, and archaea, plays a role in neurological functions and behaviors. Preliminary data from our lab suggests that oral administration of the probiotic and commensal bacteria Lactiplantibacillus plantarum (L. plantarum) may modulate locomotion and sleep in wild-type Drosophila melanogaster. We hypothesize that L. plantarum modulates behavior in flies through the Gamma-aminobutyric acid (GABA) signaling pathway. Certain L. plantarum strains have been shown to produce neurotransmitters like GABA; however, it remains unclear if the fly intestinal epithelium utilizes GABA and if L. plantarum influences this process.

This study examines the role of L. plantarum in activating GABAergic signaling in the gut and its effects on host behavior. We treated young wild-type flies with 5% sucrose (mock control), two strains of L. plantarum (wild fly-derived LpWF and cabbage-derived Lp39), and E. coli as a negative control in 5% sucrose. These treatments were administered over 3 days, and their locomotion and sleep were continuously monitored using the Trikinetics DAM2 system. Results showed that all bacteria tested slightly but significantly increased total sleep without altering sleep latency. However, only the LpWF strain of L. plantarum, known to colonize the fly gut robustly, significantly increased mean locomotor activity compared to controls.

Using the UAS/GAL4 system, we generated transgenic flies expressing the GABA receptor Lcch3, detected through Red Fluorescent Protein (RFP) expression. After feeding these flies with the bacteria and performing immunohistochemistry (IHC) on dissected guts 24 hours later, confocal imaging revealed increased Lcch3-driven RFP in the gut epithelial cells of flies exposed to LpWF. Additionally, preliminary IHC suggests that both L. plantarum strains, but not E. coli, produce GABA.

Overall, our results support the hypothesis that L. plantarum can modulate behaviors in Drosophila, produce GABA, and activate the expression of at least one GABA receptor in the gut. These findings align with a model in which this probiotic bacterium influences gut-brain communication via an intestinal-specific GABA signaling pathway. Understanding the gut-microbiota-brain axis mechanisms could lay the groundwork for probiotic-based therapeutic strategies for behavioral disorders.

410F **Entomopathogenic nematode infection modifies the *Drosophila melanogaster* larval microbiome** Sreeradha Mallick[1], Christina Pavloudi[2], Jimmy Saw[3], Ioannis Eleftherianos[3] [1]Biological Science, The George Washington University, [2]EMBRC - European Marine Biological Resource Centre, [3]The George Washington University

The *Drosophila melanogaster* microbiome consists of a diverse range of microorganisms. The microbiome is crucial for regulating physiological processes, including shaping the development and function of the host immune system. *D. melanogaster* offers distinct advantages over vertebrate models, allowing a detailed investigation of host-microbiota interactions and their effects on modulating host defense systems. The fruit fly is also an outstanding model for studying innate immune responses against parasites. Entomopathogenic nematodes (EPNs) activate immune signaling in the fly, which leads to immune responses to combat infection. However, the impact of EPN infection on the host larval microbiome remains poorly understood. Therefore, we have asked whether EPN infection affects the *D. melanogaster* larval microbiome. For this, we infected third-instar *D. melanogaster* larvae with *Heterorhabditis bacteriophora* symbiotic nematodes (containing *Photorhabdus luminescens* bacteria) and axenic nematodes (devoid of symbiotic bacteria). We have generated and used axenic EPNs in our experiments to determine the impact of the nematode alone on the *D. melanogaster* microbiome in the absence of the symbiotic bacteria. Analysis of the *D. melanogaster* microbiome reveals statistically significant differences in microbiome composition between uninfected and EPN infected larvae. We find 259 unique species absent in the EPN infected larvae compared to the uninfected controls. Also, we find that over 40 bacterial species are depleted in both symbiotic and axenic EPN infection compared to uninfected larvae. Infection with axenic nematodes further results in a significant shift in the *D. melanogaster* larval microbiome, leading to an increase in bacterial diversity compared to larvae infected with symbiotic nematodes (660 unique species compared to 401, respectively). This finding suggests that the absence of the EPN symbiotic bacteria creates ecological niches for unique species, resulting in a potentially more diverse microbiome in *D. melanogaster* infected larvae. Our research will enhance the understanding of microbial species within the *D. melanogaster* microbiome that regulate homeostasis during nematode infection. These insights could be beneficial in developing innovative strategies for managing agricultural pests and disease vectors.

411F **Characterization of the Lamellocyte Membrane Lipid Composition** Kristen Latour[1], Yong Li[2], Eric Potma[2], Catherine Brennan[1] [1]Cal State University Fullerton, [2]Chemistry, UC Irvine

Lamellocytes are a specialized *Drosophila* immune cell type that encapsulate and melanize invaders too large to be engulfed, including eggs laid by parasitoid wasps. Lamellocytes are produced following immune challenge, and, consistent with their encapsulation role, they are enormous (up to 80 µm diameter), although very thin. To better understand their unusual morphology and membrane structure, we are probing lamellocyte plasma membrane composition using both mass spectrometry and Raman spectroscopy. Raman is a powerful tool for visualizing the spatial distribution of lipids at subcellular scales. However, the use of Raman for analysis of cell membranes has been largely confined to artificial membranes: analysis of membranes of real cells has been challenging due to the high “noise” level from cytoplasmic components. Lamellocytes, with their flat shape and minimal cytoplasm volume, present an exciting opportunity to apply Raman towards the analysis of membrane lipid composition of actual cells. We will report our findings from combining the powerful tools of both lipidomics and Drosophila genetics towards the membrane lipid analysis of these unusually tractable cells.

412F **A Genome wide association screen for *D. melanogaster* genes that determine fly preferences for lactic acid bacteria** Andrew T Call Cell Biology and Physiology, Brigham Young University

Understanding Drosophila feeding and choice preferences offers valuable insight into the genetic and environmental factors that shape dietary behaviors in animals, making it a powerful model for studying how genetics influence food selection. This study reveals genetic factors underlying Drosophila melanogaster preference for beneficial gut bacteria, offering new insights into how host genetics influence dietary preferences for lactic acid bacteria.

Previous studies have defined myriad genes involved in fly feeding behaviors and preferences. In this study, we characterized fruit fly genes that contribute to the flies' preference to consume Lactic Acid Bacteria (LAB), common inhabitants of the fruit fly gut, from their diets. We first screened a laboratory CantonS fly line for its preference to choose between diets inoculated with different types of microorganisms. Among these, we found that the strongest preference was manifest in flies choosing between diets inoculated with LAB and uninoculated diets, using a FlyPAD device to measure fly feeding preferences. Then, we screened ~100 lines from the Drosophila Genetic Reference Panel for their preference to feed on LAB vs. uninoculated diets. Most, but not all, lines preferred to consume diets inoculated with LAB. Then, we performed a genome wide association (GWA) to identify candidate fly genes responsible for this phenotype. We identified several candidate genes that contribute to these LAB preference phenotypes. We are currently working to validate the GWA predictions and have confirmed at least two genes that determine flies' elevated preference for LAB. Together, these results provide insight into the genetic basis of microbiome-influenced feeding behaviors in fruit flies. By characterizing the genes associated with a preference for Lactic Acid Bacteria, we also enhance our understanding of how host organisms can be genetically predisposed to select specific microbial partners through dietary choices. This work contributes to a broader understanding of the genetic basis for host-microbiome interactions.

413F **Probing the Chemical Changes in the *Drosophila* Phagosome** Oscar David Hernandez[1], Alva Duenas Alvarez[1], Caitlin Harris[2], Catherine Brennan[3] [1]California State University Fullerton, [2]Dept Microbiology, Immunology, Molecular Genetics, UCLA, [3]Biological Science, California State University Fullerton

Macrophages are white blood cells that serve as the first line of defense against infectious microorganisms in both *Drosophila* and mammals. Macrophages engulf microbes into phagosomes, which evolve microbicidal interiors. We are using live *E. coli* expressing ratiometric GFP variants (redox-sensitive roGFP2 and pH-sensitive pHluorin) and quantifying the kinetics of their excitation spectra to establish a timeline for the chemical changes in the phagosome that follow engulfment. In mammals, acidification and Reactive Oxygen Species (ROS) are understood to play phagosomal killing roles, and it has been assumed that macrophages of the *Drosophila* use these chemical species in the same manner. However, unlike in mammals where ROS production is largely confined to the phagosome, we have found *E. coli* in the *Drosophila* blood experience significant ROS before engulfment. We are investigating whether the source of the extracellular ROS is from macrophages or another tissue. Additionally, we found that *E. coli* experience macrophage-derived ROS both in the phagosome and also while bound to the surface. This latter ROS production appears to intensify during the hours after infection. Although there is some heterogeneity in ROS levels, this supports the notion that *Drosophila* macrophages get activated in a manner that upregulates their ROS producing capabilities, which we are testing using loss-of-function approaches. Furthermore, we plan to investigate the pH kinetics and function using pHluorin and loss-of-function approaches.

414F **What Happens When Flies Eat Their Greens? Exploring diet-driven variation in fly life history traits and microbiota composition** L›Amat D Rosales, Sarah J Gottfredson Morgan, Brittany S Burnside Plant and Wildlife Sciences, Brigham Young University

We sought to better understand how diet and microbiota interactions shape fundamental aspects of health, development, and adaptability. We examined how different fruit and vegetable diets impact the microbiota composition and life history traits of *D. melanogaster* flies reared under varying microbial treatments. The treatments were axenic (bacteria-free), conventional (natural microbiome), and gnotobiotic (inoculated with specific bacterial strains) flies. Then, to determine how the diets influenced the phenotypes of the flies under these conditions, we measured fly development times and starvation resistance, two readily-measured traits that reflect the flies' life history. We also sequenced the flies microbiota, which is known to influence their life history traits. Fly development times varied with diet and microbial treatment, with axenic flies showing slower development rates, while conventional and gnotobiotic flies showed diet-dependent differences in development time. Many conventional flies developed faster than gnotobiotic flies on several diets, suggesting a microbiome-driven effect on life history traits. There were also significant diet and microbial treatment influences on fly starvation resistance. Studying the microbiota using 16S rRNA sequencing showed that each diet also shaped the microbial community composition, and identified microbial amplicon sequence variants that varied significantly in response to diet.

These results show that the combination of the different diets and microbial treatments resulted in distinct changes to microbial community composition as well as development and starvation resistance. Taken together, our results support the expectation that diet-microbiota interactions are important determinants of fly fitness. They also emphasize that feeding on different diets is likely to have consequences on the flies' adaptive responses to their wild environments.

415F **Bacterial central metabolism genes mediate *in vitro* interactions between members of the *Drosophila melanogaster* microbiota** Hyrum Pech Microbiology and Molecular Biology, Brigham Young University

The fruit fly *Drosophila melanogaster* is a model to study host-microbe interactions. These interactions are important to study, as the microbiota can have a strong impact on the fly's life strategies, activity levels, and life span. Acetic acid bacteria (AAB) and lactic acid bacteria (LAB) are the major members of the fruit fly microbiota. While a substantial body of literature already exists in this space, we were interested to see if we could use a plate-based assay as a proof-of-concept for rapidly screening pairwise interactions between community members in the fly microbiota. Previous work has established that AAB and LAB have a syntropic relationship, where both AAB and LAB benefit from the nutrients produced by the other. In particular, we are interested in the genetic mechanisms by which AAB can benefit from LAB-derived metabolites. This nutrient uptake can be seen on agar plates, where AAB lawns have increased growth – which we have named "cysts" – around LAB inoculations. However, we have found cases of AAB-LAB interactions where these cysts are not present in the AAB lawn. In this study we aim to find which *Acetobacter* genes are necessary for these bacteria to make cysts in the presence of LAB. We have found that knocking out central metabolism genes – namely *sdhA*, *sdhB*, *cysH*, *ppdK*, and *aarC* – not only lead to the lack of cyst formation in the mutant strains, but also significantly reduce bacterial growth rate. However, we have found that these mutant strains are not significantly deficient in colonizing fly guts, demonstrating that phenotypes on plates do not recapitulate in-fly interactions, and suggesting that interactions with the host can suppress certain growth deficiencies in the microbiota. Together, these findings expand our understanding of the microbe-microbe interactions in the fly gut microbiota, which are a model for understanding interactions between members of more complex communities.

416S **Environment and diet shape the geography-specific Drosophila melanogaster microbiota composition** Joseph T Gale[1], Rebecca Kreutz[2], Sarah J. G. Morgan[1], Brittany Burnside[1], Aubrey Cluff[1], John M Chaston[1] [1]Brigham Young University, [2]Plant and Wildlife Sciences, Brigham Young University

Geographic and environmental variation in the animal microbiota can be directly linked to the evolution and wild fitness of their hosts but often appears to be disordered. Here, we sought to better understand patterns that underlie wild variation in the microbiota composition of Drosophila melanogaster. First, environmental temperature predicted geographic variation in fly microbial communities better than latitude did. The microbiota also differed between wild flies and their diets, supporting previous conclusions that the fly microbiota is not merely a reflection of diet. Flies feeding on different diets varied significantly in their microbiota composition, and flies sampled from individual apples were exceptionally depauperate for the Lactic Acid Bacteria (LAB), a major bacterial group in wild and laboratory flies. However, flies bore significantly more LAB when sampled from other fruits or compost piles. Follow-up analyses revealed that LAB abundance in the flies uniquely responds to fruit decomposition, whereas other microbiota members better indicate temporal seasonal progression. Finally, we show that diet-dependent variation in the fly microbiota is associated with phenotypic differentiation of fly lines collected in a single orchard. These last findings link covariation between the flies' dietary history, microbiota composition, and genetic variation across relatively small (single-orchard) landscapes, reinforcing the critical role that environment-dependent variation in microbiota composition can play in local adaptation and genomic differentiation of a model animal host.

417S **Investigating the Role of Gut Specific *Relish* Expression in Aging in *Drosophila melanogaster*** Maryam Mukhtarov, Richard Meisel University of Houston

The gut microbiome, comprised of a diverse community of bacteria within the digestive tract, plays a vital role in maintaining an animal's health. However, as animals age, their microbiome makeup can fluctuate, leading to a state called "dysbiosis" which has previously been linked to a shorter lifespan. The immune system, particularly antimicrobial peptides (AMPs), plays a critical role in preserving gut microbiome homeostasis. In the fly *Drosophila melanogaster*, the transcription factor *Relish* regulates AMP production, and its expression in the fat body has previously been shown to impact lifespan. However, the effect of *Relish* expression within the gut remains unexplored. This study aims to examine whether modifying *Relish* expression in the gut leads to changes in the lifespan of *D. melanogaster.* To achieve this, we are utilizing two genetic approaches, CRISPR gene activation (CRISPRa) to increase *Relish* levels throughout gut cells through physiologically realistic expression and Gal4-UAS for targeted overexpression within gut cells, allowing for enhanced *Relish* levels restricted to the gut. In this ongoing study, Drosophila are being reared under controlled conditions with experimental groups subjected to *Relish* overexpression using the two approaches, alongside control groups with unaltered *Relish* expression. We will compare lifespans across these groups to assess if increased *Relish* in the gut impacts aging. By examining how gut specific AMP regulation by *Relish* influences longevity, we will gather more knowledge into the connection between immune response, gut microbiome health, and aging.

418S **FlyCAR: A CAR-Macrophage Model in Drosophila melanogaster** Junan Zhu[1], Jason Xie[1], Barbara Jusiak[2] [1]University of California Irvine, [2]Physiology & Biophysics, University of California Irvine

Chimeric Antigen Receptors (CARs) are repurposed membrane receptors that redirect cellular responses toward a novel ligand by replacing the extracellular domain of a receptor with single-chain antibody derivatives. Engineered CAR-T cells have achieved great success against blood cancers; however, their use against solid tumors is challenging, partly due to their limited infiltration into the tumor microenvironment. As an alternative, phagocytic CAR-Macrophages as a treatment against solid tumors have gained attention in recent years because macrophages can infiltrate solid tissues better than T cells. However, the tumor microenvironment can reprogram macrophages into a pro-tumor state, rendering them ineffective. Therefore, more studies on how CAR-Macrophages interact with the tumor are needed to circumvent the pro-tumor reprogramming for more efficient therapeutics. Here, we proposed to use Drosophila as a model organism to study such interactions. We constructed a Chimeric Antigen Receptor for Drosophila (FlyCAR) by combining an anti-GFP nanobody with the transmembrane and intracellular domain of the Drosophila scavenger protein Draper, a homolog of mammalian Megf10 expressed in macrophages. In parallel, we created a membrane-bound GFP and CD8 fusion protein (GFP::CD8) to act as a model of a tumor-specific surface antigen. Using regular and imaging flow cytometry and time-lapse confocal imaging, we observed that Drosophila S2 cells transfected with FlyCAR displayed elevated phagocytosis frequency towards target cells expressing GFP::CD8, and we did not observe significant off-target phagocytosis against S2 cells expressing intracellular GFP. This highlights the evolutionary conservation between Draper and Megf10 and opens up the possibility of studying CAR-Macrophages in Drosophila in vivo. We are currently conducting experiments to assess FlyCAR phagocytic efficiency using the Drosophila efferocytosis reporter pHlorina. New transgenic fly lines are also being constructed to test whether expressing FlyCAR in Drosophila hemocytes can rescue tumor-related host mortality in vivo.

419S **Hemocyte Response during *Drosophila* Ovarian Tumor Growth** Minh Q Le, Alexandra Chasse, Kimberly McCall Boston University

Most human tumors stem from epithelial cells which are found in all multicellular animals. We use the tractable model system *Drosophila melanogaster* to investigate cancer models and their effect on immune cells *in vivo*. Using the UAS-Gal4 system, we conditionally expressed the Ras^{V12} oncogene in early epithelial follicle cells to induce tumor growth in *Drosophila* ovaries. Ideally, immune cells will respond to tumor growth, though in reality, tumors often have ways to evade the immune system. Hemocytes, the professional macrophages of *Drosophila* that engulf apoptotic cells and debris, are rarely seen past the epithelial sheath surrounding the ovary as follicle cells typically engulf germline debris. However, hemocytes are observed at the germarium (near the stem cells) and near the lateral oviducts that connect each ovary to the uterus.

We found that hemocyte localization in the *Drosophila* ovary is drastically affected by the presence of tumors. Compared to the fed *lacZ* control group, fed Ras^{V12} flies had an increased number of hemocytes located on the epithelial sheath. Previously, our lab has shown that hemocyte localization near the oviduct is likely due to their role in engulfment of the corpus luteum and dead egg chamber debris. In starved vs. fed *lacZ* flies, we saw a significant increase in the number of hemocytes present in the lateral oviduct area. However, Ras^{V12} flies did not show a change in the number of hemocytes in the lateral oviduct area between the starved vs. fed groups. Upon further quantification, we observed a major increase in the number of hemocytes located in the germarium in starved Ras^{V12} flies compared to all other groups. This suggests that there is a recruitment mechanism occurring in the starved Ras^{V12} flies that localizes hemocytes to the germarium. Ongoing work is aimed at investigating the role hemocytes play in tumor development and survival.

420S **Inside *Drosophila Melanogaster's* Fight to Survive, Only One Move, the Innate Immune System** Destinee Biyoudi-Monthe[1], Ayla Preble[2], Aaidah Fathima Nizamudeen[2], Sasha Stoddard[1] [1]Biology, George Mason University, [2]George Mason University

Immunology is the study of the immune system's ability to recognize and respond to pathogens, a critical defense mechanism for all organisms. This research investigates the immune response in *Drosophila melanogaster*, a model organism that uses the Toll and Immune Deficiency (IMD) signaling pathways to defend against infections. The Toll pathway is activated by Gram-positive bacteria and fungi, while the IMD pathway responds to Gram-negative bacteria. Both pathways trigger immune responses, including the activation of chemokines, cytokines, and antimicrobial peptides, to combat pathogens.

Most studies on *Drosophila* immunity focus on the genes involved in each pathway, but limited research exists on the interaction between the Toll and IMD pathways. This project aims to fill this gap by investigating how exposure to different pathogens affects immune responses and identifying genes linking these pathways. Specifically, the project will expose *Drosophila* melanogaster to *Escherichia coli* and *Candida glabrata* on test strains containing GFP or LacZ visual indicators to detect pathway activation. We aim to validate that *E.coli* spiked fly food activates the Toll pathway, indicated by GFP expression in larval fat bodies, while *C. glabrata* spiked media activates the IMD pathway through LacZ/X-Gal assays.

Research has shown that the Dorsal protein plays a key role in the Toll pathway by controlling immune genes. When the Toll receptor is triggered, it causes the breakdown of Cactus, allowing Dorsal to enter the nucleus and initiate immune responses. Cactus may link the Toll and IMD pathways by regulating NF-kB, a protein needed to activate genes for immune responses, inflammation, and cell survival. Knowing this information, this study also aims to quantify protein expression levels of Dorsal and Cactus in various *Drosophila* strains, including wild-type and mutants of *Spz, IMD, DREDD, and Diap2*, after pathogen exposure and control conditions using Western blot analysis.

The results of the study will be used in future immunology lab courses at George Mason University to allow students to conduct a semester-long project on a gene of interest. They will decide to either perform fat body dissection and analyze cDNA-qPCR expression levels or conduct protein extraction and analyze protein levels with specific primary antibodies. The goal is for students to analyze their data and present how their gene of interest plays a role in the IMD vs. Toll signaling pathways.

421S **Role of L-2HG in Drosophila innate immune response** Mandkhai Molomjamts, Hongde Li, Jason Tennessen Indiana University

L-2-hydroxyglutarate (L-2HG) metabolism is emerging as a key regulator of cellular responses to oxidative stress, with implications for both physiological and pathological conditions. While L-2HG is synthesized in response to oxidative stress and is a part of normal cellular metabolism, it is also linked to renal carcinomas and L-2HG aciduria, a rare neurodegenerative disease. In addition, recent studies in mammalian cell lines found ectopic accumulation of L-2HG in activated immune cells and increased macrophage polarization in L-2HG treatment. These findings suggest that L-2HG plays a regulatory role in healthy tissues, underscoring the need for further in vivo studies to explore how abnormal L-2HG accumulation contributes to human disease.

Drosophila melanogaster larvae serve as an excellent model organism for studying the role of L-2HG in normal physiological conditions. Drosophila larvae synthesize and accumulate high levels of L-2HG during development through a tightly regulated process. The regulatory pathways controlling L-2HG metabolism are conserved between Drosophila and mammals, suggesting a critical role for L-2HG in cellular metabolism. Our lab previously used a mutant line lacking the L-2HG degradation enzyme, L-2HG dehydrogenase (L-2hgdh), to examine gene expression changes in this mutant compared to a control group using RNA sequencing (RNA-seq). This analysis revealed that the *Drosophila* immune response pathway is significantly altered in the absence of L-2hgdh, which aligns with findings from mammalian cell line studies.

Building on our RNA-seq results, we used a Lac-Z reporter assay to examine the expression of antimicrobial peptides (AMPs) between the L-2hgdh mutant and control groups. AMPs are part of humoral immune response of *Drosophila* and controlled through Toll and IMD pathway. We used a fly line in which the β-galactosidase (β-gal) encoding Lac-Z gene is fused to the Diptericin A (DptA) promoter, an AMP synthesized through the IMD pathway. The intensity of X-gal staining in the L-2hgdh mutant group was lower than in the control group, indicating reduced β-gal expression and, consequently, decreased DptA expression. Overall, our results suggest that L-2HG interferes with immune signaling, leading to downregulation of the innate immune response in *Drosophila*.

422S **Detection of bacteria through taste receptors primes the cellular immune response through a non-canonical Immune Deficiency Pathway** Alix Najera Mazariegos[1], Gérard Manière[2], Darius Camp[3], Rhea Kaul[3], Carla Duval[3], Romane Milleville[4], Martine Berthelot-Grosjean[2], Georges Alves[5], Julien Royet[4], Yael Grosjean[2], Pierre-Yves Musso[2], Guy Tanentzapf[3] [1]Cellular and Physiological Sciences, The University of British Columbia, [2]Centre des Sciences du Gout et de l'Alimentation, [3]The University of British Columbia, [4]IBDM, [5]Université de Bourgogne

Animals use their sensory system to detect environmental cues that they then communicate, process, and integrate through their nervous system in order to elicit a specific response. Taste cues are critical to maintain homeostasis in an organism and are important to mediate aversive behaviours to tastants linked to potential pathogens. However, to date, the links between the sensory system and the response to pathogenic threats remain poorly understood. The IMmune Deficiency (IMD) pathway is a conserved NF-κB immune signalling pathway, responsible for detecting and responding to bacterial infections by promoting the expression of antimicrobial peptides. In *Drosophila,* immune cells produced by the larval hematopoietic organ, the lymph gland, express Peptidoglycan Receptor proteins (PGRPs) that directly bind to peptidoglycan in the bacterial cell wall to activate this pathway. Here we show that *Drosophila* larvae can also use their taste system to detect bacterial peptidoglycans in their environment and respond by modulating the activity of their cellular immune system. Interestingly, we show that activation of a non-canonical IMD pathway in aversive taste sensory neurons, relies on specific PGRPs upstream the cascade to regulate immune cell differentiation in the larval lymph gland. These results demonstrate that, in animals, sensory inputs such as taste play an important role enhancing the cellular response against potential pathogens. Overall, our findings add to the growing list of examples of crosstalk between the nervous and immune systems and provide novel and important mechanisms for linking them.

423S **Using Drosophila Video Tracking to Analyze the Effects of the Microbiota on Activity** Cooper Johnson Genetics, Brigham young University

The gut microbiome has been shown to have a profound effect on its host organism, impacting not only metabolism, but even development time and lifespan. The purpose of this project is to use a new method to determine the relationships between Drosophila melanogaster activity and social interactions, and its microbiota. Previous research in this area has usually used activity monitors to record data involving activity and locomotion, and has shown that the microbiota can significantly impact fly activity. In this work we instead use Drosophila Video Tracking (DVT) software , which is capable of collecting 74 different metrics that analyze both movement and social behavior. To record data 4-6 flies are placed in a small stage which is then placed under a camera. The flies are recorded for approximately 30 mins, after which the video can be fed through an analysis pipeline which tracks each fly's individual movements and compares them to extract data points on the individual movement and behavior of each fly. Using DVT we have shown that there are significant differences in the activity of axenic (bacteria-free) and gnotobiotic (reared with a defined microbiota) flies, confirming a microbial contribution to fly activity can be identified using this software. For example, we have shown that axenic flies tend to interact with fewer other flies and tend to turn and zigzag more as they move, while gnotobiotic flies tend to have more interactions with other flies and tend to move in straighter tracks. We are currently working to screen the influence of ~ 40 different bacterial strains on fly activity as a prelude to performing bacterial genome-wide association to predict bacterial genes that control fly activity Together, these approaches will expand our understanding of the mechanistic basis for host-microbe interactions, and provide insight into specific host movements and behaviors that are influenced by their associated microorganisms.

424S **Caught red handed: Parasitoid eggs absorb host lipids** Meagan Ash, Todd Schlenke Entomology, University of Arizona

Parasitoids are insects that have a unique life history strategy. They lay their eggs on or in a host arthropod, and their offspring consume the host to acquire all of the energy/nutrients they need to emerge as adults. Many parasitoids eat very little as adults and thus have limited energy stores to provision the next generation of eggs with yolk. However, their eggs dramatically increase in size once they are laid inside the host, presumably by absorbing host nutrients via an unknown mechanism. The fruit fly *Drosophila melanogaster* is a genetic model organism that is naturally infested by a variety of parasitoid wasp species. By feeding flies fluorescent lipids, I found that parasitoid eggs from four different genera rapidly acquire host lipid stores. Using the genetic tools available in *Drosophila*, I am investigating the underlying mechanism by which this happens and whether I can genetically manipulate fly lipid physiology to control the outcome of fly-parasitoid interactions.

425S **Establishing *Drosophila melanogaster* as a Model of Invertebrate Immune Priming** Emily Burke[1], Todd Schlenke[2,3] [1]Genetics, University of Arizona, [2]Entomology, University of Arizona, [3]University of Arizona

Unlike vertebrates, invertebrates lack a lymphocyte-based adaptive immune system. There is no known mechanism by which the invertebrate innate immune system can acquire immunological memory against a pathogen. Yet, numerous studies have shown that when invertebrate organisms are exposed to a pathogen, they can acquire immunity against that pathogen such that they are able to mount an enhanced, and often very specific and/or long lasting, immune response upon subsequent exposure. This phenomenon, known as "immune priming", has been observed in a wide diversity of invertebrate organisms against numerous types of pathogens. The immunological community is encumbered by conflicting evidence surrounding immune priming. Despite efforts to understand how invertebrates acquire immunity, no single mechanism has been directly confirmed, and suggested mechanisms may not be translatable between species. *Drosophila melanogaster* is a well-established model organism that offers a multitude of readily available genetic tools and methods for studying innate immunity. This work utilizes *D. melanogaster* to investigate immune priming, as well as the specificity of acquired protection against systemic bacterial infections over time. This work contributes to scientific understanding about the mechanistic basis of immune priming. In the future, we might leverage these mechanisms to our advantage by artificially boosting immune defenses of organisms beneficial to human society, such as livestock, crop plants, and pollinators.

426S **How do bacteria-dependent dietary temperatures influence the cold-temperature behavior of Drosophila melanogaster?** Chandler Sefcik, Eliza Beales Brigham Young University

The microbiota of Drosophila melanogaster influences a wide range of the fly's phenotypic traits, including developmental rate, starvation resistance, and social behavior. In previous work, we observed that D. melanogaster reared with one strain, Acetobacter DmW_125, was more active and had lower mortality below 70°F than two other bacterial strains (another Acetobacter strain and a Weissella strain). We performed follow-up experiments on these three strains to show that the strain that led to higher fly activity and survival at low temperatures increases the temperature of the surface of the fly diet, relative to ambient temperatures. To better understand the heat production phenotype, we performed follow-up experiments that show that heat production is specific to the diet in our assay and is not observed when the microorganisms are reared under different growth conditions or on different media, including standard laboratory media for Acetobacter. Following these findings, we are working to characterize the influence of these three bacterial strains on cold-temperature traits of the flies in order to better understand the effect that bacterial heat production could have on the flies. These assays include analysis of the flies' recovery from chill coma, and their activity and social interactions at low temperatures. Together, these findings will improve our understanding of species-specific influences of the microbiota on their host, including correlation between their effect on dietary temperatures and traits that are important for activity at low temperatures.

427S **The microbiota's response to adaptive evolution of Drosophila melanogaster life history traits** Zachary Greenspan[1], Kathy Thien[2], John Chaston[3], Parvin Shahrestani[2] [1]Ecology and Evolutionary Biology, University of California, Irvine, [2]California State University, Fullerton, [3]Plant and Wildlife Sciences, Brigham Young University

Experimental evolution has, in the past decade, made significant and robust scientific progress on understanding the overall evolutionary patterns and mechanistic processes from genomic data: at first from short-read sequencing, and currently now from long-read sequencing. However, one piece critically underexplored is the role of microbiota in evolutionary history and potential host-genotype interactions. In this research we explore the microbiome from *Drosophila melanogaster* populations with four distinct aging patterns ranging from short lifespans to long lifespans. By analyzing colony forming unit abundances for acetic and lactic acid bacteria as well as metagenomic data from preexisting genomic datasets, we have investigated this missing component in evolutionary research. Our finding suggest statically significant differentiation between selection regimes; populations with different aging patterns also feature unique microbiota profiles. Additionally, there are connections between the relative abundance of Wolbachia and overall lifespan where longer-lived populations feature higher relative abundances of Wolbachia than shorter-lived populations.

428S **Treatment with Tetracycline and Rifampicin antibiotics improves survival of Flock House virus infection in young and old *Drosophila melanogaster*** Justin McGee, Dean Bunnell, Maddie Buhl, Grace Milas, Stanislava Chtarbanova The University of Alabama

Host metabolism and immunity are intricately linked in both health and disease. Aging can significantly influence this interplay, often leading to increased susceptibility to infections. Identifying interventions that mitigate the age-associated decline in immune function and improve survival outcomes from viral infections is therefore of primary importance. Prior work in our lab has shown that following infection with an RNA virus (Flock House Virus, FHV), aging *Drosophila* display stronger regulation of genes whose products function in metabolic processes and mitochondrial respiration. Additionally, oxygen consumption rate measurements used as a proxy for metabolic rate and mitochondrial function, identified significant age-dependent changes linking decreased metabolic rates to improved survival. This led us to hypothesize that modulation of host metabolism could affect survival outcomes of FHV infection and can represent an intervention for improving outcomes in older flies. To test this, we treated young (5 days-old) and aged (30 days-old) FHV-infected flies with two antibiotics that disrupt mitochondrial translation (tetracycline, TTC) and transcription (rifampicin, RIF) respectively, causing mitochondrial dysfunction. A dose-response assay testing different concentrations of TTC mixed with fly food showed that in comparison to vehicle-treated flies, 0.05mg/mL TTC significantly improved survival of FHV in both young and aged flies ($P<0.0001$). Similarly, young and aged FHV-infected flies that were fed 500mg/L RIF significantly outlived their vehicle-fed counterparts ($p<0.0001$ and $P<0.001$, respectively). Non-significant differences in survival of FHV in both age cohorts were observed when flies were fed an antibiotic, ampicillin, that did not interfere with the function of the mitochondria. To assess the impact of TTC- and RIF- treatments on viral load, we measured viral titers using RT-PCR. However, no significant differences in viral load were observed between age groups or treatment groups. Our findings suggest that antibiotic treatment targeting mitochondrial function can mitigate FHV susceptibility in both young and old flies, although further investigation is needed to fully understand the underlying mechanisms.

429S **Sleep-dependent clearance of brain lipids by peripheral blood cells** Bumsik Cho, Diane Youngstrom, Samantha Killiany, Camilo Guevara, Amita Sehgal University of pennsylvania

Sleep is essential for maintaining physiological homeostasis and influences processes such as memory, longevity in animals. While sleep is primarily regulated by the brain, recent evidence suggests that peripheral tissues also contribute to sleep regulation. Among these, immune cells are known to influence sleep, but most studies have focused on conditions of sleep deprivation or immune activation. In this study, we investigated the role of immune cells in regulating sleep under normal physiological conditions using the *Drosophila* model. We discovered that macrophage-like blood cells (hemocytes) take up lipid droplets from glial cells in a sleep-dependent manner via the scavenger receptor Eater. Disruption of lipid droplet clearance through hemocytes leads to their accumulation in glial cells, which is accompanied by increased mitochondrial oxidation in the brain and reduced NAD+ levels. Thus lipid transfer reduces metabolic stress and promotes brain integrity. These findings highlight the importance of sleep in maintaining metabolic homeostasis in the brain and reveal a novel role for circulating immune cells in this process.

430S **Deciphering infection mechanisms of contemporary African Zika Virus strains in *Drosophila* midgut** Dani OSMAN, Elodie Marguerite, Chaker EL KALAMOUNI PIMIT Laboratory

The Zoonotic Zika virus (ZIKV) is a mosquito-borne virus that emerged in 2007 in Micronesia, subsequently causing significant outbreaks in the South Pacific, the Americas, and Southeast Asia. ZIKV is a member of the Orthoflavivirus genus and is primarily transmitted by *Aedes* mosquitoes. In addition to mosquito transmission, ZIKV can also be transmitted vertically, leading to congenital syndromes in newborns. The virus circulates under different genotypes, notably the historical African and Asian lineages, with the latter responsible for recent outbreaks. Interestingly, contemporary African strains have been reported to be more efficiently transmitted by mosquitoes and to cause more severe symptoms compared to Asian strains.

In mosquito vectors, ZIKV particles initially proliferate in the midgut before disseminating throughout the body to reach the salivary glands. This process involves overcoming local physical and cellular barriers and intestinal immunity. Due to limitations in existing molecular and genetic tools, new models are required to investigate host-ZIKV interactions at the midgut level. The fruit fly *Drosophila melanogaster*, which shares similar intestinal cellular composition with mosquitoes, provides an effective model system for studying ZIKV-host interactions. The current study relies on the use of *Drosophila* to investigate at the mechanistic level how midgut barriers modulate the infectious activity of contemporary African ZIKV strains. A comparative study will be specifically conducted between two Zika virus strains isolated in Africa (2011 and 2018) and a Brazilian strain from 2015.

431T **The Genomics Education Partnership: A Community of Practice Empowering Faculty to Provide Course-based Undergraduate Research Experiences Authors** Norma A Velazquez Ulloa[1], Martin G Burg[2], Justin DiAngelo[3], Lisa Kadlec[4], Judy Leatherman[5], Wilson Leung[6], Hemlata Mistry[7], Alexis Nagengast[7], Chinmay P Rele[8], Katie M Sandlin[8], Rebecca Spokony[9], Stephanie Toering Peters[10], Laura K Reed[8], The Genomics Education Partnership[8] [1]Biology, Lewis and Clark College, [2]Grand Valley State University, [3]Penn State Berks, [4]Wilkes University, [5]University of Northern Colorado, [6]Washington University in St. Louis, [7]Widener University, [8]The University of Alabama, [9]Baruch College, CUNY, [10]Wartburg College

The Genomics Education Partnership (GEP; thegep.org) is a nationwide Community of Practice (CoP), with 265 faculty from 213 institutions, which aims to facilitate equity in undergraduate biology education. The GEP provides students with experiential learning and research opportunities in genomics and bioinformatics via Course-based Undergraduate Research Experiences (CUREs). Since 2019, more than 14,100 students have engaged in the GEP curriculum. GEP institutions include Community Colleges, Primarily Undergraduate Institutions, Minority-Serving Institutions, and Research Universities. In 2019, the GEP implemented a distributed leadership model to provide opportunities for members to participate in virtual and in-person Regional Node events and national meetings, contribute to working groups (e.g., develop new curriculum and assessment instruments), and engage in leadership roles in Committees. After initial training, the GEP members receive ongoing professional development opportunities to enable implementation of GEP CUREs in their classrooms. GEP membership grew substantially during the COVID-19 pandemic, as the GEP curriculum was ideal for engaging students in research virtually. During those years, new GEP members trained online, unlike earlier cohorts who trained in person. Recent findings on the GEP CoP show robust integration of the new members that trained virtually. In response to the pandemic, the GEP also created a highly successful Virtual Peer Mentor program to provide students and faculty additional live support on their research.

Within the GEP, students participate in large-scale comparative genomics research projects by learning how to use bioinformatics databases (e.g., NCBI, FlyBase) and tools (e.g., Genome Browser, BLAST) to construct gene models in eukaryotic genomes. Their models evaluate multiple sources of evidence, computational (e.g., alignments, gene predictions) and experimental (e.g., RNA-Seq data). Current GEP science projects focus on the evolution of genes involved in the insulin signaling and oxidative stress pathways, venom proteins in parasitoid wasps, Muller F Element genes in Drosophila, and genes associated with eggshell development in the Puerto Rican parrot. The GEP supports publication of gene models with students as lead authors and also publishes studies on science education.

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432T ***Developing Scientists*: An Interinstitutional Developmental Biology CURE and Regional DB Symposium increases student interest in research careers** Mardelle Atkins[1], Adriana Visbal[2], Sharmin Hasan[1], Elda Rueda[2] [1]Biological Sciences, Sam Houston State University, [2]Natural Sciences, University of Houston Downtown

Increased student access to research practice is a pillar of Vision and Change for increased biological literacy. Yet, not all students can access classical mentored research experiences. A way to expand access to research opportunities is course based undergraduate research experiences (CUREs). For the past 4 years, we have implemented a novel CURE approach. In Fall '21, we began a collaboration between Sam Houston State University and University of Houston-Downtown students to share analyses, results, and ideas. Here, we not only utilize the high-impact CURE practice, but also try to capitalize on building a peer community to improve STEM identity and positively impact student retention. Specifically, we use Zoom and on-line file sharing technologies so that students at the two institutions collaborate on a common problem, presenting to each other in a "lab meeting" setting. Our aim is to create a space for students to see peers as "scientists", feel belonging within a peer scientific community, and thereby increase their own STEM identity, while experiencing authentic research and engaging in a realistic remote collaborative process. Scientifically, a prior mentee performed an *in silico* screen using published transcriptomic datasets to identify 92 conserved, but poorly characterized, candidate genes for *Drosophila melanogaster* eye or head development, which our students test using RNAi knockdown and publicly available reporter stocks. With this approach, we now have a program that serves roughly 30-45 students each Fall; multiple students have presented their work at regional conferences and we have identified 4 new eye development genes which the 2024 cohort validated and furthered characterization of. In Fall '23, with Society for Developmental Biology Education Grant support, we expanded this experience by organizing a regional Developmental Biology Symposium where these students (and more) presented their research and interacted with faculty and graduate students at Baylor College of Medicine. This year institutional supports facilitated a repeat of the event. Here, we summarize preliminary qualitative and quantitative results from these interventions and their impacts on scientific identity. Student responses indicate that participation in this experience increases student scientific identity, understanding of the research enterprise, and interest in pursuit of research-oriented careers.

433T **Innate Immunity System Laboratory Class Activities Using *Drosophila melanogaster* hemocytes** Rebecca Spokony Natural Sciences, Baruch College, CUNY

Although many undergraduate biology degree programs include an immunology lecture course, most of them do not include a laboratory component. Learning complex concepts is aided with active learning. Here we describe a laboratory activity to use *Drosophila melanogaster* hemocytes to demonstrate multiple innate immunity concepts. *Drosophila* have three major hemocyte cell types, plasmatocytes that perform macrophage functions, crystal cells that perform platelet-like functions and lamellocytes that are only produced upon injury and parasitic wasp infection[1]. Many tools have been produced by the *Drosophila* research community to study these cell types. Markers for plasmatocytes include Eater and hemese, while msn is used to label lamellocytes. For these activities, we used lines with in vivo fluorescence. Since lamellocytes are only produced under various insults, we used Hop^{Tuml} with constitutively activated JAK/STAT producing lamellocytes[2]. In order to observe crystal cells, students gave the larvae 10 minute heatshocks at 37 deg C, which activates melanization within the cells. In order to observe plasmatocytes, we used both eater-GFP[3] and hemolectin-GFP[4] and msn-moCherry[5] to see lamellocytes. Using fluorescence microscopy, students could observe the distribution and quantity of these cells in whole larvae. They also examine blood composition on slides after bleeding the larvae. After learning these skills, students planned their own experiments to examine if various treatments induced an immune response. They presented posters of their results at the end of the semester. We plan to expand these activities to include various in vivo humoral response markers such as Drosomycin and Defensin, as well.

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434F **Rainbow Networks: Encouraging and retaining LGBTQ+ research trainees in STEMM** Claire Thomas Biology and Biochemistry & Molecular Biology, Penn State University

Empirical data indicates that diverse research groups produce better research outcomes. Surveys have also shown that LGBTQ+ undergraduates often try their hand at research but leave their labs at higher rates than people outside this community. Our local surveys have also indicated that LGBTQ+ students can experience feelings of exclusion from research activities (access) and communities (belonging) at significantly higher percentages than students who identify as heterosexual. Given the importance of personal connections for professional advancement in academia, especially at early career stages, establishing safe and accepting research-training environments can significantly boost retention of LGBTQ+ trainees, and improve the quality of the research enterprise.

At Penn State I developed a 'Rainbow Science Network' to develop faculty understanding of the LGBTQ+ community and to help LGBTQ+ students find research groups where the PI has made a significant public commitment to encourage people to bring their whole selves to their research group. To join the RSN, PI's must take two training workshops. The first is a general introduction the LGBTQ+ community, the second is a Transgender 101 workshop. The list of member faculty is then displayed on the College website for trainees to cross-reference with their labs of interest. The RSN at Penn State spread quickly to three other colleges (Engineering, Earth and Mineral Sciences, and Agricultural Science and Industry) and now has a total of over 100 member groups.

A favorable feature of the Rainbow Networks is that most institutions with an LGBTQ+ center already offer the required trainings, and so faculty applicant training just needs to be tracked by coordinating with such a center to obtain attendance lists. Similarly, College communications offices can implement a suitable webpage for each network at minimal to no cost. Also, there are usually well-established mechanisms for students to apply to labs and so the networks do not offer funding, nor do they offer enrollment in a lab. This is actually an important point: The networks do not require that the trainee be 'out', in fact a PI may never know that this is why a person entered their lab. While this offers some challenges for assessment, we felt that the privacy that this permits is an important feature of the system. At Penn State we did later obtain an institutional grant that allows us to extend our activities to bring in speakers as LGBTQ+ role models, hold panel discussion and try to build community across our other Commonwealth Campuses.

This model is simple to clone and customize at any willing institution.

435F **Annotating TWiM Podcast: Interactive Microbiology Learning** Suparna Chatterjee[1], Gabrielle Nicole Baca[2], Josué Benjamin Torres[2], Lauren Farrar[2], Jovani Catalan-Dibene[2] [1]Curriculum and Instruction, New Mexico State University, [2]Biology, New Mexico State University

Annotating podcast episodes of This Week in Microbiology (TWiM) as part of the MOER (Microbiology Open Educational Resource) project offered a unique and valuable opportunity for undergraduate students to work together in creating open educational resources (OER) for general microbiology topics. Students selected TWiM episodes, individually annotated episodes and then collaborated to generate a consensus annotation following an annotation template. This process focused on aligning annotations with the five core concepts outlined in Vision and Change as well as American Society for Microbiology (ASM) curricular guidelines. The premise for the experimental design is to engage students in analyzing primary literature for both snippet and main papers discussed in each podcast episode. Engaging in this active learning approach, combined with mentor guidance, fosters open discourse that enhances oral and written communication skills, while deepening students' understanding of microbiology concepts. Reflecting on the process, we realized that this technology-driven pedagogical approach offers valuable insights into alternative methods of teaching and learning to engage students' participation, sustain interest and develop competence for practicing science.

436F **Authentic research on the fly: A flexible, student-driven CURE using Drosophila melanogaster** Sarah G Clark Neuroscience Institute, Georgia State University

NEUR 2020, Introduction to Neuroscience Research, is a new CURE at Georgia State University that aims to integrate the high-impact practice of undergraduate research more fully into our Neuroscience curriculum by providing an authentic research experience before students have taken any content-based neuroscience courses.

Since there is no expectation of prior learning that the course depends on, every aspect of this CURE uses a "learn by doing" approach. Students develop a research question they are interested in, design a set of experiments to investigate it using behavioral assays and Western blotting, execute their plan, and finally analyze and present their results.

By engaging directly and organically with the research process, students begin to develop an understanding of concepts such as the importance of proper controls and the impact that choices in research design can have on results. They also experience first-hand some of the principles of neuroscience that they will later learn about in lecture courses, creating a natural foundation for this later learning.

The structure of the course is easily modified to fit various programs and institutions, and we see evidence of improved outcomes in academic and non-academic domains for students who take the course.

437S **Exploring the Impact of Cannabinoids on Ethanol Tolerance, Fertility, and Microbiome Composition in *Drosophila melanogaster*: A C.U.R.E. Approach** Alyssa M Vidal[1], Sandra Illescas[1], Mariano Loza-Coll[2] [1]Biology, California State University Northridge, [2]California State University Northridge

This study, conducted as part of a Course-Based Undergraduate Research Experience (CURE) class, examined the effects of dietary cannabinoids on female *Drosophila melanogaster*. Over the last few years, there has been a rising interest in cannabinoids like CBD and their potential health benefits, primarily related to reducing inflammation, treating gastrointestinal disorders, and preventing relapses in drug and alcohol addiction. Our research focused on investigating the impact of dietary broad-spectrum cannabinoids on three physiological parameters: functional ethanol tolerance, fertility and progeny development, and whole-fly microbiome composition. To assess functional ethanol tolerance, students performed ethanol sedation climbing assays, measuring climbing ability and the percent of active flies over time for both flies on a standard diet and a diet supplemented with cannabinoid oil (CBD diet). Most groups succeeded in generating good quality raw data obtained during the lab period (a time series of pictures of flies on a sedation cage). However, some groups struggled subsequently, when carrying out on their own a protocol for picture analysis and data quality control. After filtering out erroneous data sets, we found that a CBD diet did not significantly affect the flies' naïve resistance to ethanol, but appeared to reduce the ability of females to develop a functional ethanol tolerance upon re-exposure to the drug. To investigate the effects of maternal CBD intake on fertility and progeny development, students counted eggs, pupae, and calculated eclosion rates. Similarly, students counted different colony types growing on MRS plates to investigate the effect of a CBD diet on microbiome composition. In both cases, several groups generated unrealistically high counts, which may have resulted from their difficulties when trying to distinguish specific colony morphologies or fly eggs. Following data curation and consolidation, we found reproducible changes in the relative proportions of two distinct colony types among flies on a CBD diet. While this CURE project revealed inconsistencies and challenges in data acquisition and analysis, it also allowed us to identify proper corrective strategies for subsequent data analysis, leading to preliminary but robust insights into the effects of CBD on *Drosophila* physiology and behavior.

438S **Gene Annotation and Genomic Analysis of *Insulin-like peptide 3* (*Ilp3*) in *D. busckii*, *D. novamexicana*, and *D. subobscura*** Fady Hanna, Janelle Nelson, Hannah Sofia Nadine Rosario, Nick Reeves Biology, Mt. San Jacinto College

The Genomics Education Partnership (GEP) has provided valuable research experience to undergraduate students by involving them in gene annotation work with genomic databases. For our GEP project, we annotated the *Insulin-like peptide 3* (*Ilp3*) gene in *D. busckii*, *D. novamexicana*, and *D. subobscura* using the well annotated *D. melanogaster Ilp3* gene as a reference. *Ilp3* encodes a ligand that binds to an insulin receptor, initiating the insulin signaling pathway. The insulin signaling pathway is a fundamental component of fruit fly development and larval growth. We analyzed the genomic neighborhood around *Ilp3* in *D. busckii*, *D. novamexicana*, and *D. subobscura* and discovered that the four clustered insulin-like genes in *D. melanogaster*, *Ilp1*, *Ilp2*, *Ilp3*, and *Ilp4*, are all present. BLAST comparisons and the data tracks in the GEP Genome Browser made it possible to determine which insulin-like genes are orthologous between the species. Additionally, this region of the *D. busckii* genome contains two additional insulin-like genes that are not present in *D. melanogaster*. These additional insulin-like genes may have come from duplication of other insulin-like genes or by rearrangement of the chromosome. Based on the RNA-seq data track in the GEP Genome Browser, *Ilp3* in *D. busckii* has two isoforms created by alternative splicing that make slightly different Ilp3 proteins. This was surprising because *D. melanogaster Ilp3* only has one isoform. Analysis of the male and female RNA-seq expression patterns for *Ilp3* in *D. busckii* showed evidence that one of the isoforms is expressed in females only. Changes in the genomic neighborhood and the number of isoforms reflect the different evolutionary histories of these *Drosophila* species.

439S **Integrating Fly CURE into Anatomy and Physiology: Enhancing STEM Access for Underrepresented Students** Zully J. Villanueva[1], Alysia Vrailas-Mortimer[2], Victoria L. Straub[3], Jacob D. Kagey[4], Melanie Hwalek[3], Kayla L Bieser[5] [1]Department of Natural Sciences, Western New Mexico University, [2]Department of Biochemistry and Biophysics Linus Pauling Institute, Oregon State University, [3]SPEC Associates, [4]Biology Department, University of Detroit Mercy, [5]Department of Physical and Life Sciences, Nevada State University

Undergraduate research experiences are crucial for fostering critical thinking, research skills, and a sense of belonging in STEM. At Western New Mexico University (WNMU), a Hispanic-Serving Institution, we have implemented a Fly Course-Based Undergraduate Research Experience (CURE) module into the Anatomy and Physiology II curriculum. This initiative provides students, primarily from underrepresented backgrounds and, aspiring healthcare professionals, with valuable research opportunities they might not otherwise encounter.

The Fly CURE module significantly enhanced students' sense of belonging in the scientific community. Participants were more likely to identify as researchers and reported satisfaction from contributing to meaningful scientific work. These outcomes underscore the potential of CUREs to address inequities in STEM education and inspire future aspirations in science.

440T **A novel *Drosophila* model of amyloid-β secretion in Alzheimer's disease reveals ferroptotic-like cell death at the earliest stage of disease progression** Rosalind Heron[1], Barbara Black[1], Robert Williams[2], Will Wood[1] [1]University of Edinburgh, [2]University of Bath

Recent FDA approvals for immunotherapy approaches targeting amyloid-beta (Aβ) refocuses attention on understanding the molecular basis of Aβ toxicity, particularly during the 20 year presymptomatic prodromal phase. To enable this, we have developed a novel *Drosophila* model that replicates the first known adaptation that occurs in the Alzheimer's brain - an overproduction of Aβ42 from neurons. This allows us to interrogate the earliest mechanistic changes initiated downstream of Aβ production in an Alzheimer's brain and investigate how later problems develop from these. We observed in the *Drosophila* embryo that within hours there is already increased neuronal death, from an overproduction of Aβ42 alone. This is followed by increased accumulation of Aβ42 at the soma and axons of neurons in larvae which is associated with cognitive deficits, namely in decision-making ability, and in pupae when the Aβ42 overproduction causes mortality. Whilst much of the early cell death appears to be apoptotic, we also observe a small number of these neurons undergoing ferroptotic-like cell death and show that the cognitive deficits are rescued by feeding the larvae iron chelators. This suggests that ferroptotic-like cell death caused by neuronal Aβ42 overproduction leads to cognitive defects, such as decision-making problems. This research supports the targeting of ferroptosis as a therapeutic for Alzheimer's disease, and suggests that use of this therapy during the presymptomatic prodromal phase could prove more beneficial.

441T **Scully acts through ecdysone for aging-related cognitive decline** Paul Rafael Sabandal, Carolyne Chepkosgei, Maya Solis, Kyung-An Han The University of Texas at El Paso

Scully (*Scu*), the homolog of human 17-β hydroxysteroid dehydrogenase 10, is linked to Alzheimer's disease (AD) as it binds to Aβ peptides and is overexpressed in the postmortem brains of AD patients. Yet there is no study demonstrating how *Scu* contributes to AD *in vivo*. To address this knowledge gap, we investigated the role of *Scu* in dementia by measuring inhibitory control and memory in *Drosophila*. We found that the *Scu*/+ flies exhibit inhibitory control deficit and memory loss in an aging-dependent manner. We also identified the mushroom body as the key neural site for the *Scu*'s role in the aging-sensitive cognitive decline. *Scu* is a multifunctional mitochondrial enzyme and is known to be involved in tRNA processing and neurosteroid homeostasis. We found no genetic interaction of *Scu* with tRNA processing molecules but strong interaction with the ecdysone pathway (a major steroid hormone in *Drosophila*) for inhibitory control deficit. Consistently, the ecdysone level diminishes with aging in the wild-type fly brain, and this decrease is worsened in *Scu*/+. Moreover, *Scu* interacts with Aβ42 or mutated Tau to aggravate impulsivity and memory deficits. Together, these findings reveal a novel mechanism by which *Scu* functionally contributes to AD and related dementias.

442T **Characterization of seizure susceptibility in a *Drosophila* model of KDM5C-associated X-linked intellectual developmental disorder** Bethany K Terry[1], Julie Secombe[1,2] [1]Department of Genetics, Albert Einstein College of Medicine, [2]Dominick P. Purpura Department of Neuroscience, Albert Einstein College of Medicine

KDM5C-associated X-linked intellectual developmental disorder (XLIDD), also known as Claes-Jensen syndrome, is a rare neurodevelopmental disorder caused by variants in the Lysine (K)-specific demethylase 5C (*KDM5C*) gene. In addition to physical growth challenges, individuals with KDM5C-associated XLIDD have been reported display a spectrum of different neurological changes, which include intellectual disability, gross and fine motor delays, emotional and behavioral changes, and epilepsy. However, although seizures have been reported in up to a third of individuals with this disorder, how alterations in KDM5C activity result in the promotion of seizures is not well understood.

Previous work in our lab has demonstrated that perturbation of KDM5, *Drosophila*'s KDM5C ortholog, can impact neurodevelopment and cognitive functioning. In this study, we utilized our *Drosophila* models to investigate the role of KDM5 in seizure susceptibility. To do this, groups of adult flies were exposed to heat or mechanical stress and then recorded for several minutes. Video recordings were then assessed to determine the number of flies that seized and the time that each fly seized for. Interestingly, we found that expression of patient-related variants of *Kdm5* increases the proportion of flies that seize in response to mechanical stress in comparison to control animals. Furthermore, we found increased seizure susceptibility and duration following neuron-specific knockdown of *Kdm5* in comparison to controls. Having established the increased propensity of our *Drosophila* model to seize, ongoing work will aim to identify the mechanisms underlying the seizures that occur following KDM5 perturbation.

443T **Use of fluorescent biosensors for the detection and characterization of physiological hydrogen sulfide in *Drosophila melanogaster*** Sunayn Cheku[1], Blase Rokusek[2], Haishi Cao[3], Kimberly A Carlson[1] [1]Biology, University of Nebraska at Kearney, [2]University of Nebraska at Kearney, [3]Chemistry, University of Nebraska at Kearney

Endogenous hydrogen sulfide is an integral component of normal cellular functioning regulating key processes, such as cell signalling and cellular stress response, and physiological processes, such as vasodilation and inflammation. Previous studies have observed a dysregulation of hydrogen sulfide mediated pathways in neurons to be a characteristic feature of neurodegenerative diseases such as Parkinson's disease (PD) and Alzheimer's disease (AD). However, studies on endogenous hydrogen sulfide activity have been limited due to lack of effective techniques to do so. This study attempts to use fluorescent hydrogen sulfide sensing compounds to detect endogenous hydrogen sulfide within PC12 cells, S2 cells, and *D. melanogaster* brain tissue. We further compare fluorescent signals between healthy Drosophila brain tissue and Drosophila PD model brain tissue to evaluate the utility of the fluorescent sensors as a potential diagnostic tool for PD. The results of this study will demonstrate that these sensors provide a robust and reliable means for detection of hydrogen sulfide in biosamples.

444T **A drug repurposing screen leads to discovery of small molecule modulators for FAM177A1-related disorder** Katherine Beebe[1], Emily Coelho[1], Caroline Massey[2], Heather Evans[2], Clement Y Chow[1] [1]Human Genetics, University of Utah, [2]University of Utah

FAM177A1-related disorder is a rare autosomal recessive disorder caused by mutations in the *FAM177A1* gene. Affected individuals show varied symptoms, typically including intellectual disability, developmental delay, macrocephaly, seizures, and gait abnormalities. *FAM177A1* encodes a polypeptide of 236 amino acids, and little is known about its function. The *Drosophila* ortholog of *FAM177A1* is *CG8300*, sharing 44% amino acid similarity. Given the limited knowledge of *CG8300* function in *Drosophila*, we first aimed to identify a suitable loss-of-function phenotype for a primary screen, with the goal of conducting a small molecule screen to identify potential therapeutic compounds. Our study identified a muscle-specific role for *CG8300*, where knockdown in muscle tissue resulted in 93% pupal lethality in male flies and a distinct half-eclosion phenotype. Using this male-specific lethality as a measurable phenotype, we conducted a small molecule screen to find compounds that modify *CG8300* loss-of-function outcomes, with the goal of discovering candidate therapeutics. Screening a library of 1,520 FDA- and EMA-approved compounds, we identified 33 suppressor compounds that improved male survival and 9 enhancer compounds that worsened it. Further studies focused on 11 clinically relevant drugs, many of which are associated with mechanisms involving acetylcholine signaling. Current studies are exploring the mechanisms by which these compounds rescue *CG8300* loss-of-function phenotypes in muscle. Additionally, we are extending our research to examine the effects of *CG8300* loss and drug rescue in glial cells, where *CG8300* loss leads to seizures.

445T **A drug repurposing screen reveals dopamine signaling as a critical pathway underlying potential therapeutics for the rare disease DPAGT1-CDG** Hans M. Dalton[1,2], Naomi J Young[2], Alexys R Berman[2], Kaylee A Patterson[2], Sydney J Peterson[2], Heather D Evans[2], Clement Y Chow[2] [1]Molecular Biosciences, University of Kansas, [2]Human Genetics, University of Utah

Glycosylation pathways add essential sugar modifications to proteins and other molecules. Deleterious mutations in glycosylation genes underlie Congenital Disorders of Glycosylation (CDGs). CDGs are ultra-rare disorders that cause debilitating, multisystemic symptoms. There are few treatment options available for CDGs and little known about the molecular mechanisms involved. Drug repurposing screens, which use libraries of FDA-approved drugs, can help find potential therapeutics and connect known drug targets to disease biology. Here I will present a completed drug repurposing screen on the rare disease DPAGT1-CDG.

Like many CDGs, DPAGT1-CDG manifests multisystemically with severe developmental and CNS disorders. It is caused by mutations in the gene *DPAGT1*, which encodes for the first essential enzyme in N-linked glycosylation. I used an eye-based model of DPAGT1-CDG in *D. melanogaster* that causes a small, rough eye phenotype. Screening with a drug repurposing library of 1,520 compounds, I identified 42 candidate drugs that rescued the DPAGT1-CDG model eye size.

The strongest drug class was dopamine receptor D2 (D2R) antagonists. Knockdown of *D2R* mimicked the drug and strongly improved eye size. D2R acts oppositely to the dopamine 1 receptor (D1R). In line with this, a heterozygous null of *D1R* worsened eye size and reduced the improvement of *D2R* knockdown. Finally, knockdown of dopamine synthesis and recycling pathways also rescued the *DPAGT1* model. Thus, the loss of dopamine flux, and its subsequent binding to D2R, improved the impairment of *DPAGT1*. In addition to dopaminergic drugs, I validated acetylcholine-affecting drugs, COX inhibitors (NSAIDs), and an ion transporter (NKCC1)-related drug, as well as a negative hit, an antihistamine. These pathways represent novel biology related to *DPAGT1*, and they may underlie new drug options for DPAGT1-CDG.

446T **Use of large-scale *Drosophila* genetic reagents to facilitate human disease research** Oguz Kanca[1,2], Grace C Burns[1], Megan Cooper[1], Ali H Bereshneh[1], Wen-wen Lin[1], Liwen Ma[1], Hirokazu Hashimoto[1], Mei-Chu Huang[1], Ming Ge[1], Toshiyuki Takano-Shimizu[3], Susan E Celnicker[4], Robert W Levis[5], Michael F Wangler[1], Shinya Yamamoto[1,6], Hugo J Bellen[1] [1]M & H Genetics, Baylor College of Medicine, [2]Duncan Neurological Research Institute, Texas Children Hospital, [3]Kyoto Drosophila Stock Center and Faculty of Applied Biology, Kyoto Institute of Technology, [4]Biological Systems and Engineering, Lawrence Berkeley National Laboratory, [5]Division of Biosphere Sciences and Engineering, Carnegie Institution for Science, [6]Department of Neuroscience, Baylor College of Medicine

The Gene Disruption Project (GDP) has generated thousands of alleles for *Drosophila* genes including MiMIC and CRIMIC T2AGAL4/KozakGAL4 (GAL4) alleles. These GAL4 insertions typically generate severe loss of function (LOF) alleles. They also allow for sensitive and accurate detection of the expression pattern of the targeted gene. In addition, the T2AGAL4 cassette can be efficiently and precisely excised by FLP recombinase which can be used to restore the function of the gene in specific tissues/cells and assess whether LOF phenotype is rescued. Hence, they allow the determination of the loss of function phenotypes, the expression pattern and the role of the gene in specific tissues. The GAL4 alleles also allow rescue of the mutant alleles with the UAS-human cDNA transgenic lines of homologous gene in about 60% of the genes. We have therefore also generated a large-scale collection of UAS-human cDNAs. To date, we created approximately 3500 GAL4 alleles and 5500 UAS-human cDNA transgenic stocks. Using these available stocks from the BDSC and the Kyoto Stock Center, we can currently test the functionality of 2055 human genes in flies. In collaboration with clinicians we have screened many variants associated with rare diseases and have contributed to the discovery of over 50 new human disease genes. In numerous cases, the analysis of the expression pattern combined with phenotypic data has led to the identification of previously unknown gene functions in specific cell populations (e.g. *Tau*, *Gba1a*, *IntS11*, *Cdkl*). By revealing some of biological processes underlying some of these rare diseases, we were able to identify drugs in flies that are effective in patients' cells (e.g. *Acox1*, *iPLA2-VIA*, *Pits*). Our studies have also revealed new pathways that are affected in common diseases (e.g. Autism, Parkinson's and Alzheimer's Disease).

We are currently increasing the size of our collections of GAL4 and UAS-human cDNA transgenic stocks. We are also developing strategies to exchange the promoter and tags of our transgenes through crosses to generate human cDNA stocks under the control of other binary systems (e.g. LexA, QUAS) and devising strategies that allow inserting the human cDNA in the locus of fly ortholog. Expanding the size and versatility of the collections will further establish *Drosophila* as a prime model organism in the human disease research.

447T **Sleep deprivation aggravates impulsivity in *Scully* mutants in an aging-dependent manner** Maya Solis[1], Carolyne Chepkosgei[1], Paul Sabandal[2], Kyung-An Han[2] [1]Biological Sciences, The University of Texas at El Paso, [2]The University of Texas at El Paso

Alzheimer's disease and related dementias are a collection of progressive neurodegenerative diseases caused by genetic and non-genetic risk factors. How these risk factors interact to cause dementia, however, remains understudied. To assess this gap in knowledge, we performed a non-biased genetic screen and identified *Scully* (*Scu*), which codes for a multifunctional mitochondrial enzyme. We found that the flies with the heterozygous mutation in *Scu* (*Scu/+*) exhibit aging-dependent memory loss and impulsivity. To identify whether the non-genetic risk factor sleep loss interacts with *Scu* for cognitive impairment, we disrupted the control and *Scu/+* flies' sleep at night via random light stimuli (LSD) for 1 day (acute) or 3 days (chronic) at three different ages: 4 day (young age), 2 weeks (mid-age), and 4 weeks old (early old age). We found that acute LSD did not increase impulsivity in control flies at 4 days old; however, acute LSD in *Scu/+* led to significantly increased impulsivity, which was further augmented with chronic LSD and aging. These observations indicate that *Scu/+* interacts with sleep deprivation and aging for impulsivity. Since both sleep deprivation and aging are shown to increase reactive oxygen species and sensitivity to oxidative stress, we explored them using redox-GFP reporters expressed in the mushroom body gamma neurons, the functional site for Scu. In the absence of aging or sleep deprivation, control and *Scu/+* flies showed similar redox homeostasis. Currently, we are assessing how sleep deprivation and aging affect redox homeostasis in *Scu/+*. Overall, our study will provide novel mechanistic insights on the genetic and non-genetic risk factor interaction for Alzheimer's disease and related dementias.

448T **Mutant variant of tRNA processing enzyme impairs motor and cognitive function in *Drosophila*** Saathvika Rajamani[1], Lucia Vilchez[1], Nicole Cracovia[1], Saul Landaverde[2], Atulya Iyengar[2], Edward B Dubrovsky[1] [1]Biological Sciences, Fordham University, [2]Biological Sciences, University of Alabama

Emerging evidence from clinical studies has identified mutations in genes encoding tRNA processing enzymes as contributors to neuropathology. Among these is *RNase Z*, that codes for an endoribonuclease responsible for cleaving off the 3' trailer of mitochondrial and nuclear primary tRNA transcripts. Human patients harboring mutant variants of this gene exhibit a spectrum of neurological dysfunctions, yet a direct causal connection remains to be established. To investigate RNase Z associated neuropathology, we used CRISPR/Cas9 technology to generate fly models expressing a known pathogenic mutation, Thr494Ile, in a pan-neuronal and neuronal mitochondrial-specific manner. Our previous analyses in these mutants revealed aberrant mushroom body phenotypes, and reduced length and branching of the motor neurons innervating the flight muscle. Since these anomalies are observed in neurons involved in cognition and flight respectively, we hypothesized that both of these functions might be negatively affected in the mutant *RNase Z* flies. To this effect, we assessed integrity of the giant fiber mediated escape circuit by monitoring electrophysiological activity in flight muscle, DLM or dorsal longitudinal muscle. We observed a significant retardation in DLM latency and an inability of the circuit to follow high frequency stimulation, thereby indicating neurotransmission defects. Further assessment revealed a progressive decline in the flight performance of the mutants, with markedly reduced landing height averages even at a younger age. Rapid decline in short and long-term memory formation or retrieval was observed when cognition was evaluated with olfactory learning assays. Collectively, our data indicates that expression of mutant *RNase Z* results in neuropathological phenotypes and thereby successfully establishing a causal connection between the two. Notably, the deterioration in motor and cognitive functions was comparable in both the pan-neuronal and neuronal mitochondrial mutants, suggesting that mutant RNase Z expression specifically in neuronal mitochondria, is a primary instigator of the associated neurodegenerative phenotypes. Thus, we report here the first animal model of neuron-specific mutant *RNase Z* exhibiting impairments in both motor and cognitive abilities.

449T **Mechanisms of neurofibromin-mediated modulation of metabolic homeostasis** Valentina Botero, Seth Tomchik University of Iowa

Neurofibromatosis type 1 is a genetic disorder with a metabolic component, though the underlying mechanisms are unclear. Neurofibromatosis type 1 is a multisystemic disorder, arising from loss-of-function mutations in a single gene, *NF1*. This gene encodes neurofibromin (Nf1), a large protein with a central GAP-related domain (GRD) which modulates numerous cellular and molecular processes. Clinically, individuals with neurofibromatosis type 1 present with a spectrum of symptoms, including brain and peripheral nerve tumors, neurocognitive and behavioral deficits, and skeletal and vascular abnormalities. In humans, Nf1 is implicated in metabolic regulation, as individuals with neurofibromatosis type 1 exhibit a reduction in stature and body mass index, pituitary growth hormone deficiencies, muscle weakness, and increased insulin sensitivity. However, despite indications of Nf1's involvement in metabolic regulation, the molecular mechanisms underlying these metabolic effects remain unknown.

To investigate the link between Nf1 and metabolism, we leveraged the *Drosophila* model of neurofibromatosis type 1, which recapitulates key aspects of the disorder and shares highly conserved signaling pathways. Our *in vivo* genetic analysis revealed that the loss of Nf1 impairs metabolic homeostasis, resulting in heightened metabolic and feeding rates, disrupted lipid dynamics, and increased sensitivity to starvation. These metabolic effects map to a distinct set of interneurons in the nervous system, which can be dynamically modulated to elevate metabolic rate. Further investigation revealed that Nf1 primarily regulates metabolic rate via neuronal mechanisms, with additional contributions from muscle cells.

Neurofibromin's Ras GAP activity is modulated by its central GRD, and our research identified Ras signaling as a key factor in mediating Nf1's effects on metabolism. Our *in vivo* genetic experiments targeting multiple signaling molecules suggested that two Ras-dependent signaling pathways are critical in metabolic regulation, with Nf1 orchestrating metabolic control through their coordinated activity. Additionally, Nf1 deficiency did not alter neuronal mitochondria number or structure. These data reveal a novel interaction between Nf1 and metabolism, delineating the neural circuits and signaling pathways responsible for Nf1-metabolic regulation.

450T **Comparison of phenotypes caused by homozygous and heterozygous *blm* deficiency in Drosophila** ERGUL SUSAMCI[1], Keith A Maggert[2] [1]GENETICS, The University of Arizona, [2]Molecular and Cellular Biology, The University of Arizona

The *Bloom* DNA helicase is a member of the RecQ family of ATP-dependent helicases. The *Drosophila* ortholog, *blm*, is encoded by the *mus309* locus. Absence in humans gives rise to Bloom›s Syndrome (BS), a rare, autosomal recessive disorder characterized by sterility and predisposition to many different cancers. BS cells express extreme genetic instability characterized by chromosomal breaks and sister chromatid exchanges. People with Bloom Syndrome also express proportional dwarfism that as-yet has no clear explanation.

We created a complete deletion of all 4 exons of the *Drosophila blm* gene (the blm^{ES} allele). We obtained mutant flies with different genotypes (blm^{ES}/ blm^{ES}, blm^{ES}/ *+*, *+/+*) using the blm^{ES} allele and compared their phenotypes at both cellular and tissue/organ levels. We discovered that while blm^{ES}/ blm^{ES} flies escape the embryonic lethality caused by the absence of the *blm*gene, adult mutants lack the capacity to produce new offspring. When comparing the overall body weight, wing width and depth between homozygous (blm^{ES}/ blm^{ES}) and heterozygous (blm^{ES}/ *+)* mutants, we discovered that homozygous flies had smaller bodies and wings than heterozygous siblings. Although it is known that *blm* deficiency causes genomic instability, the reason for this growth retardation has not been determined. Currently, there are two main competing ideas: (i) that *blm* deficiency leads to a lengthened the cell cycle time, which in turn causes a decrease in the number of cells, and (ii) that increased DNA damage increases the rate of apoptosis in mutant cells. The blm^{ES} allele closely recapitulates the human small size phenotype, allowing usto address these ideas, as well as other persistent gaps in the study of *Bloom* function.

Our work has led us to a third hypothesis, (iii) that the relationship between *blm* and *rDNA (*ribosomal DNA) contributes to the small size phenotype. Analysis of BS clinical entities and patient-derived cell lines show a defect preferentially in hard-to-replicate DNAs including *rDNA*. *blm* enhanced the bobbed *rDNA* deficiency phenotype, mostly through a persistent *blm*-mediated loss of *rDNA* copy number. This reduction was attended by cytological defects in nucleolar structure. *blm* mutants also produced rare magnified *rDNA* arrays, indicating *blm* mutation generally destabilizes chromosome at the *rDNA*. We determined *rDNA* copy numbers by qPCR in 3 different human cell lines as well as in blood samples collected from Bloom syndrome patients. Mutations in the *blm* gene cause hypervariability in the *rDNA* copy number, as they did in *Drosophila*. Our data suggest that *blm* stabilizes *rDNA*, and defects lead to hypervariability – usually appearing as loss, but occasionally appearing as gains – in *rDNA* copy number. We will continue to investigate if and how this instability contributes to the small size phenotype in both humans and *Drosophila*.

451T **Effects of Long-Term Nicotine Exposure on Adult *Drosophila melanogaster*** Avi Strok[1], Blake Tellinghusen[2], Jessica Naworski[2], Norma A Velazquez Ulloa[3] [1]Biochemistry and Molecular Biology, Lewis & Clark College, [2]Biology, Lewis & Clark College, [3]Biology, Lewis and Clark College

Tobacco use continues to kill millions of people every year worldwide, with e-cigarette use especially on the rise among young people. Nicotine is the addictive chemical in tobacco products, and continued use results in increased tolerance and reliance. Despite knowledge of the receptors nicotine acts on and some mechanisms of action downstream of nicotine, treatments for tobacco addiction remain only partially successful. More research into novel genetic mechanisms of nicotine's effects could identify targets for new treatments and elucidate the mechanisms behind addiction-related behaviors. *Drosophila melanogaster* is a model organism with a proven record to identify novel mechanisms of action for drugs of abuse, and previous research showed similar responses to mammals during developmental nicotine exposure, including decreased survival rate and decreased sensitivity to nicotine and ethanol in adulthood.

The aim of this investigation was to characterize the effects of nicotine exposure treatment in the w^{1118} strain of adult female and male flies on dosage-dependent survivorship, locomotor activity in a negative geotaxis assay, sensory perception in an olfactory function assay, and oviposition preference in an egg-laying assay. As expected, nicotine negatively affected survivorship. 5-day nicotine exposure decreased locomotor activity but had no effect on olfactory perception. Female flies show significant egg-laying preference for nicotine-laced substrate, regardless of prior nicotine exposure. Nicotine exposure significantly reduced the total number of eggs laid by female flies.

This initial characterization of multi-day nicotine exposure in a control strain of young-adult flies lays the groundwork for the identification of nicotine resistant and sensitive phenotypes. We are now repeating these experiments on mutant fly strains found to be resistant to developmental nicotine exposure. Moving forward, we are quantifying nicotine concentration in fruit flies using high performance liquid chromatography. By doing so, we will determine if nicotine metabolism is comparable between Drosophila and humans to further validate our insect model.

452T **Functional analysis and classification of rare genetic variants in *SATB2* using *Drosophila melanogaster* and patient-derived iPS cells** Hirokazu Hashimoto[1,2], Radhika Padma[3], Samantha L Deal[4], Oguz Kanca[1,2], Kenji Yokoi[5], Shinya Yamamoto[1,2] [1]Molecular and Human Genetics, Baylor College of Medicine, [2]Jan and Dan Duncan Neurological Research Institute, Texas Children's Hospital, [3]Biology, University of Dayton, [4]Howard Hughes Medical Institute and Chronobiology and Sleep Institute, Perelman School of Medicine, University of Pennsylvania, [5]Research Center for Child Mental Development, Kanazawa University

SATB2-associated syndrome (SAS) is a rare genetic disorder with over 750 known cases worldwide. This syndrome is characterized by developmental delay, intellectual disability, and craniofacial dysmorphisms with variable expressivity and penetrance. This gene encodes for an evolutionarily conserved DNA binding protein that regulates chromatin remodeling. Because genotype-phenotype relationships in SAS have not been clearly established, we assessed the functional consequences of disease-associated variants in *SATB2* using fruit flies. We generated transgenic flies that express human *SATB2* under the control of the GAL4/UAS system and compared the phenotypes induced by ubiquitous or tissue/cell-type specific ectopic overexpression of reference or variant proteins *in vivo*. We studied two nonsense variants and 13 missense variants (6 variants in CUT1, 5 variants in CUT2, and 2 variants in HOX domains) previously reported in SAS patients. We found that overexpression of reference and variant human SATB2 in the wing, dorsal thorax, eye, neuron, or glia of *Drosophila melanogaster* permits the classification of disease-associated genetic variants. First, two nonsense variants behaved as strong loss-of-function (LOF) alleles. Second, the early CUT1 variants behaved as milder LOF alleles compared to the nonsense variants. Third, most CUT2 variants behaved primarily as gain-of-function (GOF) alleles. It is interesting to note that some variants showed both LOF and GOF properties, indicating that these variants have functional effects that are context dependent (which we refer to as 'mixed' alleles). In addition, to assess neural function, we performed electroretinogram analysis. These results are consistent with the overexpression analysis, classified as LOF, GOF and mixed alleles. Moreover, we investigated the patient-derived iPS cells from a patient carrying the mixed allele. After the forebrain neuron's differentiation, we found that the neuron increased the number of branching and spine-like structures. These phenotypes may be affecting the patient›s neural symptoms.

In conclusion, some *SATB2* variants found in SAS patients behaved as LOF alleles, whereas others behaved as GOF variants or with mixed properties of LOF and GOF. Understanding the functional consequence of each patient's variant has clinical implications because therapeutic design should be different for patients with LOF variants (e.g., gene therapy) and GOF variants (e.g., antisense oligonucleotide).

453T **Functional studies of missense variants in *ZDHHC15* identified in patients with neurodevelopmental diseases using *Drosophila*** Mei-Chu Huang[1], Oguz Kanca[1], Lorenzo Botto[2], Michael Kruer[3], Hugo J Hugo Bellen[1], Michael Wangler[1], Shinya Yamamoto[1] [1]Baylor College of Medicine, [2]University of Utah School of Medicine, [3]Phoenix Children's Hospital

ZDHHC15 (*zinc finger DHHC-type palmitoyltransferase 15*) is one of 24 genes in the human genome that encodes a DHHC (aspartate-histidine-histidine-cysteine) motif-containing enzyme implicated in protein prenylation. This gene is located on the X chromosome and earlier human genetic studies suggested its involvement in intellectual disability. More recently, a male patient with multiple neurological symptoms including hypotonic cerebral palsy, focal-onset epilepsy, cortical visual impairment, intellectual disability, autism spectrum disorder, anxiety, and aggressive behaviors was found to carry a maternally inherited rare missense variant (p.H158R) in this gene. Through the Undiagnosed Diseases Network (UDN), we identified a female patient with multiple neurological symptoms including global developmental delay, febrile seizures, and absence epilepsy who carries a different *de novo* missense variant (p.F224L). While the missense variant identified in the male patient was shown to function as a strong loss-of-function (LOF) allele through studies performed in yeast, the functional consequence of the female patient's variant is unknown. Furthermore, while studies in flies, zebrafish, and mice suggest this gene is necessary for the proper development and/or function of the nervous system, the precise molecular function of ZDHHC15 *in vivo* is ill-defined.

To determine whether flies can be used to study the function of disease-associated ZDHHC15 variants, we generated transgenic *Drosophila melanogaster* strains that express reference or variant human ZDHHC15 based on the GAL4/UAS system. We found that ubiquitous overexpression of reference ZDHHC15 causes lethality, and tissue-specific overexpression causes various morphological defects. The p.H158R variant ZDHHC15 lacks this activity, consistent with this variant being a LOF allele. Interestingly, ZDHHC15 with the p.F224L variant causes stronger phenotypes when overexpressed compared to the reference protein, suggesting this is likely a gain-of-function. We further performed co-overexpression experiments to obtain further evidence that p.H158R is a hypomorphic allele and p.F224L is a hypermorphic allele. Using a versatile *T2A-GAL4* line, we found that the fly ortholog of *ZDHHC15* (*CG1407*) is expressed in a subset of larval and adult brain neurons, and its loss causes semi-lethality. Together, these data provide evidence that both loss and gain of function variants in *ZDHHC15* likely cause neurodevelopmental disorders in humans.

454T **Alterations in morphology and synaptic function identified in a *Drosophila* model of a rare exon duplication in *PHACTR1*.** Jonathan Andrews[1], Paige Hall[1], Sharayu V. Jangam[1], Lauren C. Briere[2], Rebekah E. Townsley[1], Shinya Yamamoto[1], David A. Sweetser[2], Michael Wangler[1] [1]Molecular and Human Genetics, Baylor College of Medicine, [2]Undiagnosed Diseases Network, Massachusetts General Hospital

Phosphatase and Actin Regulator 1 (*PHACTR1*) is a significant contributor to cytoskeletal organization, cell morphology, and cell motility. Multiple *PHACTR1* variants have been previously associated with different human pathologies, including developmental and epileptic encephalopathy, West syndrome, myocardial infarction, melanoma growth, coronary artery disease, and cervical artery dissection. Through the efforts of the Undiagnosed Diseases Network (UDN), we identified a human proband with a *de novo* duplication of exon 5 and parts of the surrounding introns. *Drosophila CG32264*, which is orthologous to human *PHACTR1*, is highly expressed in the anterior midgut primordium, the embryonic and larval midgut, the posterior midgut primordium, and the nervous system of the adult fly. We have generated a *Drosophila* model of three *PHACTR1* variants, including our proband's variant and two missense mutations previously identified as causal for West Syndrome. Our overexpression studies have demonstrated significant differences in survival between our variant and the West Syndrome variants when ubiquitously overexpressed via Actin-, Tubulin-, or Daughterless-Gal4 drivers. Furthermore, differences in the morphology of the wing and notum could be observed when our variants were overexpressed via Nubbin- or Pnr-Gal4 drivers, with more significant alterations observed with the west-syndrome associated variants. Uniquely, ubiquitous overexpression of the proband's variant was sufficient to induce forking or abnormal bends in bristles. These changes within the actin-rich bristle structures were not observed in animals overexpressing the reference human cDNA or CG32264. To identify changes in cytoskeletal organization, we performed a series of flip-out experiments to acutely express reference and variant *PHACTR1* in a subset of cells within the salivary glands. We identified significant changes in the expression of tubulin within our variant, while salivary glands expressing west-syndrome associated variants, our human reference line, or fly CG32264 did not display alterations in tubulin level. As one of the primary phenotypes associated with west syndrome is epileptic encephalopathy, we performed a series of electrophysiology experiments using the larval neuromuscular junction (NMJ). These experiments demonstrated that flies overexpressing our variant had significant changes in the amplitude and frequency of responses from the NMJ, while control animals did not. Our results clearly indicate that our *Drosophila* model is capable of identifying important changes resulting from *PHACTR1* variants and that duplications of the 5th exon have a mechanism of pathogenesis separate from variants previously associated with West syndrome.

455T **The Developmental and physiological impacts of pathogenic human HTT in the nervous system** Tadros Hana[1,1], Veronika Mousa[1], Alice Lin[2], Andrew Michael[1,3], Madona Aziz[1], Sevinch Kamardinova[1], Sabita Bansal[1] [1]Biology, Middle Tennessee State University, [2]Biology, Brown University, [3]Middle Tennessee State University

Huntington's disease (HD) is a neurodegenerative disease that falls under the umbrella of inherited polyglutamine (polyQ) disorders. HD affects approximately 1 in 20,000 people yearly, manifesting as a progressive decline in motor control, cognitive deterioration, and psychiatric disturbances. The pathophysiology of HD has been linked to the formation of insoluble aggregates caused by aberrant truncation of the huntingtin protein (htt) when the polyQ region is expanded. The aggregates have been shown to accumulate in the cytoplasm and the nucleus of neurons. To investigate the mutant htt aggregation phenotype and its associated physiological dysfunctions, we expressed two constructs of the human HTT in the Drosophila nervous system, one with a pathogenic polyQ expansion (P-htt) and one with a non-pathogenic version (NP-htt), both fused with an N-terminal RFP for in vivo visualization. Fluorescence microscopy of these animals revealed that P-htt aggregates began accumulating in the larval ventral nerve cord cell bodies as early as 24 hours post egg-hatching, with profound axonal aggregation observed by the 48-hour timepoint. Synaptic and Dense core vesicle trafficking was significantly impaired both up- and downstream of P-htt aggregates, supporting evidence of p-htt's dysregulation of the molecular motor machinery while simultaneously acting as a physical blockade when aggregated. Surprisingly, no ultrastructural alterations to the motor axons' innervation length, synaptic structure, or their postsynaptic muscle targets were observed. However, severe reductions in muscle-force output were recorded, with muscle contraction kinetics resembling chorea in humans. Consequently, crawling in third-instar larvae showed marked reduction in all locomotion metrics measured. Additionally, we established a novel aggregation model based in the Drosophila adult wing that reflected the aggregation proliferation pattern recorded in the larval nervous system. Expressing P-htt in the adult nervous system resulted in early lethality that was partially rescued by supplementing the food media with mTOR inhibitor Rapamycin. Taken together, these findings elucidate the timeframe of aggregate formation as well as the downstream physiological implications on the nervous system and its peripheral tissue targets. The multifaceted exploration of HD herein serves as a key step in further understanding the development, pathology, and treatments of the disorder to potentially ameliorate and eventually cure the symptoms of those suffering its effects.

456T **Establishment of a *Drosophila* model for a new progeria-lipodystrophy disease caused by *BUD13*** Mikiko Oka[1,2], Oguz Kanca[1,2], Michael Francis Wangler[1,3], Hugo Bellen[1,3], Shinya Yamamoto[1,3] [1]Molecular and Human Genetics, Baylor College of Medicine, [2]Jan & Duncan Neurological Research Institute (NRI), Texas Children's Hospital, [3]an & Duncan Neurological Research Institute (NRI), Texas Children's Hospital

Progeria syndromes (PSs) are a group of rare genetic disorders that cause premature aging. As exemplified by Hutchinson-Gilford Progeria Syndrome caused by *LMNA* variants, some PSs show abnormal nuclear envelope (NE) and lipodystrophy. A recent clinical study has identified patients with a homozygous *BUD13* variant presenting global developmental disorder, lipodystrophy, and progeroid features, including invaginated NE. BUD13 is a component of REtention and Splicing (RES) complex, which regulates splicing introns. Although all five patients have the same variant producing a truncated protein, two patients with milder symptoms express a short isoform that was not expressed in control individuals. However, how these alleles affect BUD13 functions and contribute to these phenotypes is unclear.

To analyze phenotypes in fly *Bud13 (CG13625)* loss of function (LoF) and how variants in human *BUD13* affect protein function, we generated CRISPR-mediated knock-out/knock-in fly with *Kozak-GAL4* and transgenic flies that express the human reference or variant human *BUD13* cDNA using the UAS/GAL4 system. We revealed that *Bud13*is expressed in multiple tissues, and important for development in multiple tissues. From human cDNA overexpression experiments, we concluded that the BUD13 truncation observed in all patients is a LoF allele. In contrast, a short isoform seen in two patients with milder symptoms still possessed function, suggesting that this allele may contribute to mild phenotypic presentation in the two individuals.

Next, we asked whether knockdown of *Bud13* recapitulates patients' phenotypes, such as lipodystrophy and NE alterations. Fat body-specific knockdown reduced fat body tissue and lipid droplet storage indicating the important role in fat body homeostasis. Also, abnormal NE was observed in fat body- and muscle-specific knockdown. Since *Bud13* was strongly expressed in the ring gland, we investigated the functions in this tissue. Prothoracic gland-specific knockdown showed developmental arrest at 3rd instar larvae indicating ecdysone deficits. To investigate molecular mechanisms in LoF animals, we performed RNA-seq using muscle-specific knockdown, and are currently focusing on mitochondria and oxidative stress pathways.

457T ***Drosophila* models of sporadic and genetic Parkinson's disease** Angeline Claudia Atheby, Katarzyna Dominika Rosikon, Hakeem Lawal Delaware State University

Parkinson's disease (PD) is a debilitating neurodegenerative disease. The precise cause of most of its cases remains unknown despite decades of research that have established key environmental and genetic factors as contributors to its etiology. Moreover, notwithstanding those advances in our understanding of the disease, a viable treatment remains elusive. Here we are interested in using two model systems, rotenone an environmental toxin, and alpha synuclein (α-synuclein) a Parkinson's disease gene, to advance our understanding of the disease. Rotenone is a potent laboratory model for sporadic PD that has been used to uncover important insights into the etiology of the disease. Here we aim to test the neuroprotective capability of the small molecule dacarbazine (which we identified in a previous pharmacological screen) and its structural derivative, 5-Amino-4-imidazolecarboxamide (AICA) against rotenone induced neuronal toxicity. Both compounds have been reported previously to increase synaptic activity in a manner that is dependent on vesicular monoamine release. In this project, we investigated whether both compounds are capable of conferring organismal and/or neuroprotection against rotenone toxicity. We report that dacarbazine confers a small but reproducible protection against organismal toxicity induced by rotenone exposure in both male and female *Drosophila.* These results are all the more remarkable given that dacarbazine is a chemotherapeutic drug with a toxic potential of its own. We also report for the first time that consistent with its published role as a VMAT-dependent drug, AICA protects dopamine (DA) neurons against rotenone-induced neuronal toxicity in an assay in which we combined both a pesticide (rotenone) and age as risk factors for PD. Additionally, to test the efficacy of dacarbazine's protective capacity, we have developed an α-synuclein model in *Drosophila*. α-synuclein is the first identified genetic risk factor for PD and a key component of Lewy Bodies whose formation is one of the pathological hallmarks of the disease. We report here the generation of a myc::RFP tagged α-synuclein transgenic line. We showed that while there is no major impact in survival, the overexpression of this construct leads to a significant reduction in locomotor ability in age dependent manner. Taken together, these data suggest a promising new anti-PD compound and a platform to test its efficacy in a genetic model of this disease.

458T **Metabolism and lifespan of Drosophila melanogaster after smoke exposure regulation following Wnt pathway activation** Liu Yang, Jan-Philip Kühle, Judith Bossen, Roeder Thomas Zoological Institute, CAU Kiel

Metabolism and lifespan of *Drosophila melanogaster* after smoke exposure regulation following Wnt pathway activation

Liu Yang[1], Jan-Philip Kühle[1],Judith Bossen[1,2],Thomas Roeder[1,2]

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Chronic obstructive pulmonary disease (COPD) is the leading cause of mortality and morbidity worldwide and is characterised by incomplete reversibility of expiratory airflow limitation, dysregulated chronic inflammation and emphysematous destruction.The WNT signaling cascade controls a myriad of biological phenomena throughout the development and adult life of all animals.The regulation of WNT signaling in the development of COPD/emphysema by cigarette smoke exposure leads to dysregulation of classical and non-classical dysregulation of the WNT signalling pathway, which leads to oxidative stress, apoptosis/proliferation, inflammation, mucus hypersecretion, protease/anti-protease imbalance, autophagy, senescence, metabolic reprogramming, mitochondrial dysfunction or altered stem/progenitor cell renewal.

All these cellular processes are involved in the pathogenesis of chronic bronchitis or emphysema through increased lung injury and impaired lung repair. We used a simple cigarette smoke-induced *Drosophila* model of COPD based on chronic cigarette smoke exposure that encapsulates the main pathological features of the disease and can therefore be used to investigate new therapeutic strategies. We used this model to examine the role of WNT signalling in COPD and showed that overexpression of WNT/β-catenin reversed the premature death, reduced physical activity, reduced body fat and protein, increased metabolic rate and reduced tolerance to hypoxia caused by Chronic cigarette smoke exposured. While many studies have focused on the typical WNT/β-catenin protein signalling pathway, recent reports have highlighted that the atypical WNT signalling pathway may also have a significant impact on chronic lung disease. These findings provide potential therapeutic targets for intervention in COPD/emphysema.

459T **The analysis of the humanized Drosophila model of the Orc6 based Meier- Gorlin syndrome mutation reveals an unexpected molecular mechanism** Maxim Balasov, Katarina Akhmetova, Igor Chesnokov Biochemistry and Molecular Genetics, University of Alabama at Birmingham

Meier-Gorlin syndrome (MGS) is a rare autosomal recessive disorder characterized by microtia, primordial dwarfism, and skeletal abnormalities. Patients with MGS often carry mutations in genes encoding the subunits of subunits of the Origin Recognition Complex (ORC), components of the pre-replicative complex and replication machinery. Orc6 is an important subunit of ORC and has functions in both DNA replication and cytokinesis. Approximately 30% of the reported ORC6 related cases have compound heterozygosity in the *ORC6* gene (c.2T>C(p.Met1Thr)/c.449+5G>A). Mutation c.2T>C disrupts start ATG codon and thought to initiate at downstream in frame Methionine (amino acid number 20). The c.449+5G>A mutation causes an in-frame exon skipping. Molecular lesions caused by either mutation suggest the translation of the significantly truncated ORC6 proteins. In this study, we used our previously designed humanized Orc6 based fly model to investigate this case of MGS in live organism. We further demonstrated that truncated proteins fail to rescue *orc6* deletion, which contradicts mild clinical phenotype observed for patients with this variant. To resolve this discrepancy, we placed strong Kozak sequence, which is naturally present in human *orc6* mRNA, in front of mutated start codon in the human-*Drosophila* Orc6 transgene. The resulting construct rescued *orc6* deletion, and survived flies demonstrated phenotype that we observed earlier for other MGS mutants in *Drosophila*. These results strongly support a hypothesis that patients with mutation of the start ATG (c.2T>C) rely on full size ORC6 protein initiated from non-canonical ACG codon.

460T **Microbial mediation of Drosophila lipid metabolism** Joshua T Derrick[1], Haolong Zhu[2], Steven A Farber[2], William B Ludington[2] [1]Biology, Johns Hopkins University, [2]Biology, Johns Hopkins University/Carnegie Embryology

Since the industrial revolution, diets in the western world have undergone significant transformations, becoming much richer in saturated fats and sugars and relatively poorer in fiber and micronutrients. In addition to increasing rates of heart disease, obesity, and diabetes, these dietary changes have been accompanied by a shifting of the human gut microbiome, a collection of microorganisms that lives throughout our digestive tract. Microbial communities associated with western diets have been causally linked to worse health outcomes, although both the mechanism and whether individual species are causal remains unclear. Work in both zebrafish and mice has shown that animals with a microbiome absorb more lipids into their enterocytes from a fatty meal than germ-free animals However, a mechanism has yet to be established: how exactly do microbes promote intestinal lipid absorption.

Our lab has developed a fly model with a natural, complete, and defined microbiome consisting of seven species isolated from the gut of a single wild D. melanogaster. The goal of my project is to determine if members of this microbial community are linked to increased intestinal lipid absorption or whole animal lipid accumulation, and to establish a mechanistic link between microbial colonization and the altered lipid metabolism of the fruit fly host. I first investigated this question by using HPLC lipidomics to identify changes in whole animal lipids in the presence and absence of microbiota. These data showed shifted lipid profiles in animals colonized with bacteria. To specifically measure the postprandial response, I used the fluorescent lipid BODIPY-C12 as a metabolic tracer that was mixed into fly food containing a non-fluorescent non-absorbable dye to control for feeding rate. Using HPLC and confocal microscopy, I found that the foregut, in addition to being the major site of colonization, was the major uptake site for this lipid. To determine what genes were involved in this process, I performed post-prandial RNA-sequencing on germ-free and colonized foreguts. This generated a list of candidate genes to target with a foregut specific driver to further determine the specific pathway through which the microbes act. These findings suggest an underappreciated role for the foregut in *Drosophila*lipid metabolism, potentially as organ for sensing meal contents and absorption of simple nutrients.

461T **Different systemic impacts of Aβ42 and Tau revealed by whole-organism snRNA-seq in *Drosophila*** Ye-Jin Park[1], Tzu-Chiao Lu[1], Tyler Jackson[1], Linsey D Goodman[1], Lindsey Ran[1], Jiaye Chen[1], Chung-Yi Liang[1,2], Erin Harrison[1], Christina Ko[1], Xi Chen[1], Baiping Wang[1], Ao-Lin Hsu[2], Shinya Yamamoto[1], Yi Zhu[1], Hui Zheng[1], Yanyan Qi[1], Hugo J Bellen[1], Hongjie Li[1] [1]Baylor College of Medicine, [2]National Yang Ming Chiao Tung University

Both neuronal and peripheral tissues become disrupted in Alzheimer's disease (AD). However, a comprehensive understanding of how AD impacts different tissues across the whole organism is lacking. Using *Drosophila*, we generated an Alzheimer's Disease Fly Cell Atlas (AD-FCA) based on whole-organism single-nucleus transcriptomes of 219 cell types from flies expressing AD-associated proteins, either human Aβ42 or Tau, in neurons. We found that neuronal Aβ42 primarily affects the nervous system, including sensory neurons, while neuronal Tau induces accelerated aging in peripheral tissues, including the fat body, gut, and reproductive system. We identified a cluster of neurons enriched in Aβ42 flies, which has high lactate dehydrogenase (LDH) expression. This LDH-high cluster is conserved in the 5XFAD mouse and human AD datasets. In contrast, we found a conserved defect in fat metabolism in adipose tissue from both fly and mouse tauopathy models. The AD-FCA offers new insights into how Aβ42 or Tau systemically and differentially affects a whole organism and provides a valuable resource for understanding brain-body communications in neurodegeneration.

462T **Tumor-Invasion Dynamics at the Blood-Brain Barrier during Brain Metastasis** Chaitali Khan[1], Nasser Rusan[2] [1]Cell and Developmental Biology Centre, National Institutes of Health, [2]Cell and Developemntal Biology Center, National Institutes of Health

The tumor microenvironment (TME), composed of resident and recruited cells, is crucial for metastasis to secondary organs. While studies have examined recruited immune and stromal cells' roles, the complexity of current models limits understanding of tissue-resident cells and cell-type heterogeneity in supporting metastasis. To address this, we developed a reliable model to study metastatic tumors at secondary sites by modifying the classic allograft transplantation assay, demonstrating robust metastasis in adult *Drosophila* organs such as the brain and ovaries. This model enabled us to explore if and how a tumor breaches the blood-brain barrier (BBB) to invade the brain. Our work provides an unprecedented view of cellular dynamics between invading tumor cells and the fly BBB, which comprises perineurial glia (PNG), sub-perineurial glia (SPG), and an outer basement membrane (BM). Tumors derived from larval neural stem cells mutant for lethal-giant-larvae ($lgl^{-/-}$) were transplanted into wild-type hosts, resulting in metastasis to, and deformation of, the host adult brain. Our findings show that the tumor migrates collectively, forming an invasive front at points of contact with the brain surface. Lineage-specific labeling of the blood-brain barrier (BBB) revealed that the tumor disrupts perineurial glia (PNG) cell contacts, often causing PNG cell loss. Additionally, sub-perineurial glia (SPG) cells are pushed by the tumor but maintain intact cell-cell junctions, indicating that while the PNG layer is disrupted, SPG cells act as a barrier to tumor invasion. Genetic ablation of SPG confirmed this, as it enhanced tumor invasion and growth. Furthermore, activated macrophages or the tumor-associated macrophages (TAMs) are recruited to the invasion front, particularly where PNG cells are disrupted, and the basement membrane (BM) is damaged. This study establishes a robust *in vivo* model for real-time analysis of BBB-tumor interactions during brain metastasis, revealing differential responses of BBB cells and involvement of immune cells. Ongoing work includes electron microscopy to uncover finer BBB-tumor interactions and further genetic ablations to investigate specific BBB cell functions, TAMs recruitment mechanisms, and the role of TAMs in BBB disruption and tumor invasion.

463T **Trigger warning: Reward-less mate perception as a trigger of neurodegeneration in a *Drosophila* TDP-43 model** Narmin H Mekawy[1,2], Joshua Dubnau[1,3] [1]Neurobiology and Behavior, Stony Brook University, [2]Graduate Program in Neuroscience, Stony Brook University, [3]Anesthesiology, Stony Brook University

The most common risk factor for neurodegeneration is age. But genetic and environmental effects also are at play. For example, about 10% of amyotrophic lateral sclerosis (ALS) cases are thought to be familial, with the remainder having no genetic cause. Among the genetic forms of ALS, frontotemporal dementia (FTD) and Alzheimer's disease (AD), mutations in different genes are at play in different families. For example, a rare form of familial ALS is caused by mutations that in the TAR-DNA binding protein, TDP-43. But in the majority of ALS and about half of FTD and AD cases, abnormal cytoplasmic inclusions of TDP-43 are found in the cytoplasm of both neurons and glial cells. Thus, most cases exhibit aggregation of TDP-43 that is of wild-type sequence.

Much of what is known about the underpinnings of neurodegeneration come from animal models that utilize over-expression of TDP-43, which triggers mislocalization and aggregation that mimics many of key downstream effects. But there are no models of sporadic disease because animals such as worms, flies, mice, rats and fish do not exhibit sporadic neurodegeneration or TDP-43 aggregation as they age. Thus, the upstream forces that trigger TDP-43 protein pathology are not known. Epidemiological studies reveal strong correlation between rates of neurodegeneration and prior diagnosis with neuropsychiatric disorders such as PTSD, anxiety and depression. This suggests the possibility that psychological stressors may be upstream drivers, but it is difficult to distinguish whether depression or anxiety are pro-drome symptoms that occur before onset of neurodegeneration or are causal forces that trigger onset of neurodegenerative disease.

We are testing whether stressful percepts are sufficient to trigger spontaneous neurodegeneration in flies in which the endogenous fly TDP-43 ortholog (TBPH) is replaced with the human gene. We find that while such knock in flies exhibit normal lifespans and show no evidence of neurodegeneration, they are genetically sensitized to effects of exposure to males who have genetically feminized oenocytes. Such rewardless mate perception has previously been found to shorten lifespan of wild type males, but this effect is greatly exacerbated in males that contain the human TDP-43 knock in. We are now examining the effects of this stressful percept on physiological correlates of neurodegeneration such as aggregation of the TDP-43 gene, and activation of known downstream effectors

464T **How Parkinson's disease moves - assessing extracellular vesicles in *Drosophila melanogaster*** Allison M Johnston, Kathryn A. Jewett Juniata College

The *GBA* gene encodes for an enzyme that aids in the degradation of lipids. Without this enzyme, these lipids build up in the cell and impair function and survival. Both *Drosophila melanogaster* and humans have this gene and without it are at risk of developing Parkinson's disease and related symptoms. Previous studies have found GBA traveling throughout the flies, even in areas where it was not genotypically expressed. One mechanism for explanation is extracellular vesicles, which are membrane bound packages sent from cells to deliver substances and communicate. Extracellular vesicles isolated from flies lacking GBA have been found to contain increased protein aggregation. The aim of this study is to use primary cell cultures from third instar *Drosophila melanogaster* larvae to assess protein contents or absence within extracellular vesicles. We will also look at the abundance of extracellular vesicle proteins, being sent by different cell types, like glial cells, in vivo using adult *Drosophila melanogaster*.

465T **The influence of Jra misexpression on a *Drosophila* model of MJD** Katie R. Weispfenning[1], John Warrick[2] [1]Biology, University of Richmond, [2]Biology, Univ Richmond

Machado-Joseph Disease (MJD), also known as Spinocerebellar Ataxia type 3 (SCA3), is an autosomal dominant disorder characterized by progressive neurodegeneration resulting in early death. MJD results from a polyglutamine repeat expansion in the coding region of the *Ataxin-3* gene. MJD can be accurately modeled in *a Drosophila melanogaster* model. Jun N-terminal kinase, or JNK, is a mitogen activated protein kinase involved in regulating apoptosis, inflammation, cell proliferation, and the immune response. It is suggested that JNK may play a role in MJD and other neurodegenerative disorders, but its exact role is unknown. A downstream target of JNK in *D. melanogaster* is Jun-related antigen (Jra). To investigate the potential role of Jra in the kinase pathway response to MJD, Jra has been up and down regulated in our fly model of MJD. Preliminary evidence suggests that up regulation of Jra slows down the long term deterioration associated with MJD.

466T **Expressing disease and non-disease causing Ataxin3 in glial cells of *D. melanogaster*.** Caro A. Osenga, John M. Warrick Biology, Univ Richmond

Machado-Joseph Disease (MJD), also known as Spinocerebellar ataxia type 3 (SCA3), is an autosomal dominant neurodegenerative disease in humans. MJD is a type of polyglutamine (PolyQ) disorder caused by expansion of a 'CAG' (cytosine-adenine-guanine) nucleotide sequence repeat within the ATXN3 coding region. As a result, mutant Ataxin-3 protein misfolds and forms insoluble aggregates in the nuclei of neurons. Ultimately, this is toxic for neurons, and causes them to die. While it is known how neurons respond to mutant Ataxin-3 protein, the glial cell response has not been widely studied. Our research aims to observe the glial response to disease and non-disease causing ATXN3 expression, including aggregate formation and cell survival. Preliminary research suggests that glial cells do not die from mutant Ataxin-3 expression.

467T **Mis-regulation of the innate immune response in a *Drosophila* model for MJD** Reese A. Silberman, John Warrick Biology, Univ Richmond

Machado-Joseph Disease (MJD) is a dominantly inherited neurodegenerative disease caused by a polyglutamine repeat expansion in the Ataxin-3 gene. This places it in the Polyglutamine family of diseases with Huntington Disease and several other diseases. The formation of protein aggregates in the brain is a hallmark of the disease. Further inflammatory immune responses are seen in human neurodegenerative diseases. Previous studies have shown the immune system, particularly the NF-kB/ Relish pathway, may play a role in the neurodegenerative process of the disease. Current research suggests the disease pathology leads to activation of the innate immune pathway, leading to destruction of neurons containing protein aggregates. This study seeks to further examine the role of the NF-kB pathway in neurodegeneration utilizing a disease model in *D. melanogaster*. *D. melanogaster* has an analogous innate immune pathway, culminating in the transcription factor Relish. Relish and a protein further upstream in the pathway, IMD, were down regulated to observe the effect on neurodegeneration. Preliminary data has suggested that down regulation of the innate response influences MJD pathology.

468T **Tip 60 expression and co-localization in a *Drosophila* of Machado-Joseph Disease** Sampson S. Valdez, John Warrick Biology, Univ Richmond

Machado-Joseph Disease (MJD) or Spinocerebellar Ataxia 3 (SCA3) is an inherited neurodegenerative disease like Huntington Disease which impacts the cerebellum, brain stem, basal ganglia, spinal cord, and some cranial nerves (Matos et al. 2019). The mutated protein that causes MJD is called ATXN3 and its primary pathology involves the formation of insoluble aggregates of mutant protein, as well as other proteins, primarily in the nucleus. The aggregating mutant protein may inactivate or sequester these other proteins preventing them from functioning. The mutant protein may also prevent expression of other proteins through transcriptional dysregulation. Previous studies in our lab have demonstrated that neurodegeneration can be partially rescued by up-regulation of the histone acetyl transferase Tip60. Further, we have observed loss of Tip60 phenotype resembles mutant ATXN3 pathology in larval locomotor behavior assays. This suggests loss of Tip60 and mutant ATXN3 may act through the same mechanism. Our research looks to see if Tip60 expression is altered due to mutant ATXN3 expression or if it colocalizes with ATXN3. Using our *Drosophila* model of MJD, Western blots and immunohistochemistry were used to observe protein expression levels to look at protein localization. Preliminary data suggest mutant ATXN3 is colocalizing with Tip60 inhibiting its function.

469T **Characterizing optineurin mutation in *Drosophila* as a model of amyotrophic lateral sclerosis** Hubert Acheampong[1], Mousumee Khan[1,2], Sarah Haque[1], Ryan Insolera[1] [1]Wayne State University, [2]Ophthalmology, Visual and Anatomical Science, Wayne State University

Optineurin (OPTN) has been identified as mitophagy adaptor protein important for the autophagic removal of damaged mitochondria and protein aggregates. Mutation in the OPTN are causative for familial forms of Amyotrophic Lateral Sclerosis (ALS). Understanding how mutation in the OPTN causes ALS is crucial to fight against this fatal neurodegenerative disease. While numerous mutations in the OPTN cause familial ALS, the heterozygous E478G missense mutation is dominantly inherited, and in a highly conserved region of the protein, providing an opportunity to investigate the disease in animal models. Here, we have created a *Drosophila* model based on the neuronal expression of the fly homolog of OPTN known as kenny (key), which we mutated to create the equivalent mutation to the human E478G mutation (key^{E283G}). We found that mutant E283G protein aggregates and accumulates in larval motor neurons. Moreover, the E283G protein aggregates colocalize with and recruit the functional mitophagy machineries Atg8 and dOPTN. We hypothesize that this sequestration depletes the functional pool of mitophagy mediators causing mitophagy defects that underlies the dominant nature of the E478G human mutation. The physiological assessment of flies expressing E283G mutant protein showed substantially reduced lifespan and age-dependent climbing performance compared to flies expressing wild-type (WT) protein. Overall, this in vivo study reveals that the pathogenicity of the OPTN mutation to fly neurons represents an important step towards our understanding of the pathogenesis of human ALS.

470T **Tumor induced paraneoplastic renal defects and ascites development** Anindita Barua[1], Fei Cong[1], Hongcun Bao[1], Xianfeng Wang[1], Yi-Chun Huang[1], Yang Tang[2], Xiaowen Liu[2], Wu-Min Deng[1] [1]Biochemistry and Molecular Biology, Louisiana Cancer Research Center, Tulane University, [2]Biomedical Informatics and Genomics, New Orleans Bioinnovation Center, Tulane University

Ascites is a condition where abnormal fluid accumulation in the abdominal cavity causes complications in cancer patients and poses challenges for clinical management. In this study, we explored the role of tumor-induced gut dysbiosis and the dysregulation of renal transporter in *Drosophila* abdominal bloating. We have recently established a transplantable *Drosophila* tumor model to facilitate the study of tumor growth and host responses. Our preliminary findings indicate that these tumors trigger an innate immune response in host flies, leading to ascites-like fluid accumulation and reduced host lifespan. Our studies revealed ascites in female tumor hosts resulting from dysbiosis of the gram-negative gut commensal bacterium *Acetobacter*. Simultaneously, our mass spectrometry studies identified the dysregulation of the ion transporter of Malpighian tubules-functional unit of human kidney tubules in *Drosophila*. Investigating the signaling pathways underlying these phenomena, we aim to elucidate the potential relationship between gut dysbiosis, triggered immune responses, and perturbed ion transporters in ascites manifestation in tumor hosts. Our study underscores the critical roles of immune activation and ion transporters leading to renal damage during tumor-host interactions. This research holds the potential for identifying a novel therapeutic target to alleviate ascites in cancer patients and paving the way for improved approaches to diagnosis and treatment.

471T **Gut Bacterial Translocation Promotes Tumor-Associated Mortality by Inducing Immune-Activated Renal Damage.** Fei Cong, Hongcun Bao, Xianfeng Wang, Wu-Min Deng Tulane University

Glomerulopathy is a paraneoplastic syndrome strongly linked to poor prognosis in cancer patients. Using a novel transplanted tumor model, we observed significant damage to nephrocytes—podocyte-like cells in *Drosophila*—in tumor host flies. With the overall number of nephrocytes reducing, the filtration structure of nephrocyte was disrupted and more endosome vesicles were accumulated inside of host nephrocytes.

Meanwhile, RNA-Seq and mass spectrometry data revealed that immune pathways were activated in tumor-bearing flies at a late stage. Further investigation showed that this abnormal immune activation was driven by translocated *Acetobacter*, the most common symbiotic bacterium, which leaked from the compromised gut of the tumor host. Surprisingly, the bacteria in the body cavity triggered an immune response in nephrocytes, and this immune activation directly damaged the nephrocyte's functional structure, even in the absence of a tumor.

To confirm the impact of translocated *Acetobacter* on nephrocytes and tumor-bearing flies, we first inhibited the Imd pathway by knocking down the receptor PGRP-LC and the transcription factor Relish. This intervention reduced nephrocyte damage and extended the lifespan of the tumor-bearing flies. Additionally, our data showed that treatment with a detoxifying drug, antibiotic-added food, or germ-free conditions all increased survival time and protected nephrocyte integrity in the tumor host flies. Furthermore, when *Acetobacter* was mono-associated with the tumor-bearing flies, it induced a nephrocyte phenotype similar to that seen under normal conditions and shortened the flies› lifespan.

Taken together, we found that the innate immune response, initiated by translocated commensal bacteria from a compromised intestine, significantly contributes to reduced lifespan in tumor hosts. Our data identify the renal system as a central hub of this paraneoplastic syndrome model. The findings highlight the critical role of the gut-kidney axis in the paraneoplastic complications observed in cancer-bearing flies.

472T **Effect of Toxic Heavy Metal Mixtures on the Genome in *Drosophila* and human cells** Caitlin A Clark[1], Barbara Frederick[2], Elle McDonald[3], Tin Tin Su[3] [1]Molecular Cellular Developmental Biology, CU Boulder, [2]CU Boulder, [3]MCDB, CU Boulder

Heavy metals in the environment produce a human health risk, whether they are found naturally or are a byproduct of human industry. Due to past and current mining activity, the state of Colorado contains several contaminated sites with higher than US-average levels of lead (Pb), cadmium (Cd), and arsenic (As) in the surface soil. Pb can displace essential metals in enzymes, disrupting normal catalytic function, while also generating harmful reactive oxygen species (ROS) that cause oxidative stress and cellular damage. Cd can act as a catalyst to promote the formation of ROS. As binds sulfhydryl groups in proteins and can substitute for phosphorous in critical biochemical processes leading to the disruption of protein function and cellular energy metabolism. The genotoxic effects of Pb, Cd, and As have historically been studied in isolation (one metal at a time), yet they occur as co-contaminants in the environment. The effect of metals in combination on the genome remains poorly understood but available data suggest that one metal can influence the effect of another when both are present. To fill this knowledge gap, we are systematically assessing the effect of combinations of Pb, Cd and As on the genome of human epithelial cells and *Drosophila* larvae. Endpoints we monitor include ROS levels, cell viability and cell death, and DNA damage. In addition, we monitor longer term effects in *Drosophila* including adult fertility of larvae raised on metal-contaminated food and permanent changes to the genome in terms of Loss of Heterozygosity (LOH), using fluorescent reporters we described in recent publications (Brown et al., 2020, PLoS Genetics, PMID: 33075096 and Brown et al., Genetics, 2023, PMID: 37214983). Results from these studies will be presented.

473F **Unveiling the role of hippo interactors in glioma progression in *Drosophila* glioma model** Venkata Satya Devi Burugupalli[1], Arushi Rai[1], Amit Singh[2,3,4], Madhuri Kango-Singh[4,5,6] [1]1.Growth Regulation and Signalling Laboratory, Department of Biology, University of Dayton, [2]Drosophila Development and Disease Laboratory, Department of Biology, University of Dayton, [3]Premedical Program, University of Dayton, [4]Integrative Science and Engineering Centre, University of Dayton, [5]Growth Regulation and Signalling Laboratory, Department of Biology University of Dayton, [6]Premedical Program University of Dayton

Chronic inflammation has the potential to impact in tumorigenesis including gliomagensis. Yorkie (YAP/TAZ in human), downstream effector of Hippo pathway by interacting with its regulatory pathway such as JNK might play critical role in promoting glioma progression. *Drosophila* is employed as an *in-vivo* model for investigating to study glioma and immune regulation. Presence of diverse evolutionarily conserved cellular pathways and gene homology in *Drosophila* makes it ideal and relevant model to higher vertebrates. The preliminary data from transcriptomics, scRNA-seq in primary and recurrent GBM, and immune landscape of GBM associated microglia gain suggest that activation of NFkB and YAP lead to activation of several transcription factors that together cause cellular and signalling alterations that promote GBM growth. In *Drosophila* we intend to study the role of interactions of Yorkie (YAP/TAZ in human) with interactor pathways and the innate immune system. Here we modelled two published glioma models by constitutively activating EGFR and PI3K in one, and by knocking down PTEN with Ras overexpression in another by using Gal4-UAS expression in CNS. Here we checked for cellular markers in the established glioma models. We also investigated for inflammatory markers in GBM and immune invasion in glioma. We will present the data on role of Yorkie interactors and inflammation using immunostainings and qPCR analyses and western blot of larval brains of both wildtype and glioma models.

Keywords: GAL4-UAS, Hippo Pathway, Inflammation, Glioma

474F **The effect of a manganese porphyrin compound on brain protein nitration and nitric oxide synthase levels in parkin-null *Drosophila*** Michaela Barber[1], Amber N Juba[2], Tigran Margaryan[3], Ines Batinic-Haberle[4], T Bucky Jones[5], Artak Tovmasyan[3], Lori M Buhlman[1] [1]Biomedical Sciences Program, Midwestern University, Glendale, [2]Midwestern University, Glendale, [3]Translational Neuroscience, Ivy Brain Tumor Center at Barrow Neurological Institute, [4]Radiation Oncology, Duke University Medical Center, [5]Anatomy, Midwestern University, Glendale

Parkinson's disease (PD) is a common neurodegenerative disorder that presents with hallmark motor symptoms caused by selective degeneration of substantia nigral dopaminergic neurons. Homozygous loss-of-function mutations in the *PRKN* gene, which encodes E3 ubiquitin ligase parkin, cause a rare form of PD. Oxidative stress and impaired protein homeostasis are heavily implicated in cellular pathology of all PD forms. Nitric oxide (NO) signaling is altered in PD; however, the implications of these changes are unclear because NO promotes synaptic maintenance and inflammation in a context-dependent manner. Using nitric oxide synthase (NOS)-driven GFP expression and antibody labeling, we have observed decreased *Drosophila* NOS levels and protein nitration (an NO-mediated post-translational modification) in the central brain of parkin-null flies. Of the three mammalian NOS isoforms, the single *Drosophila* NOS isoform (dNOS) has the highest sequence homology to neuronal NOS (nNOS), which is essential for synaptic maintenance. NNOS activity is regulated by glutathionylation, and we previously reported disrupted glutathione redox equilibrium in parkin-null *Drosophila* protocerebral posterior lateral region 1 (PPL1) dopaminergic neurons, which degenerate in the absence of parkin. Here we have administered a redox-active manganese porphyrin (MnP), commonly known as mimic of superoxide dismutase, to parkin-null *Drosophila* and measured central brain dNOS and protein nitration levels. MnPs are redox-active compounds that can promote glutathionylation and improve outcomes under oxidative stress conditions. Thus, we hypothesized that administration of a highly redox-active MnP, MnTE-2-PyP^{5+}, in standard food would increase central brain dNOS and protein nitration levels in parkin-null *Drosophila*. We also hypothesized that MnP-treated parkin-null flies would have improved survival and climbing behavior. Preliminary data indicate that MnTE-2-PyP^{5+} treatment improves climbing behavior in parkin-null females and increases survival of control but not parkin-null flies. Our results will further the exploration of the interplay between parkin and NO signaling pathways. This study will also promote future work addressing the therapeutic potential of MnTE-2-PyP^{5+} in PD models.

475F **Distinguishing *PEX* gene variant severity for mild, severe, and atypical peroxisome biogenesis disorders in *Drosophila*** Vanessa A Gomez, Oguz Kanca, Sharayu V Jangam, Saurabh Srivastav, Jonathan C Andrews, Michael F Wangler Molecular & Human Genetics, Baylor College of Medicine

Peroxisomal biogenesis disorders (PBD) are autosomal recessive disorders caused by loss-of-function mutations of one of the *PEX* genes responsible for peroxisomal formation. Impaired peroxisome assembly causes severe multisystemic failure with patient phenotypes ranging from epilepsy, liver disease, feeding issues, biochemical abnormalities, and neurodegeneration. Variants in the same *PEX* gene can produce wide differences in severity, ranging from death in the first year to adults with milder complications. To study this strong genotype-phenotype correlation, we selected specific human *PEX* gene mutations and utilized *Drosophila* as a model organism. We generated fly lines in which the coding sequence of our *Pex* gene of interest has been replaced by the *KozakGAL4 (KZ)* promoter trap sequence. These cassettes simultaneously knock-out of the *Pex* gene and knock-in a *GAL4* driver, ideal for making "humanized" flies in which the human *PEX* gene can replace the fly loss. We assessed $Pex2^{KZ}$ and $Pex16^{KZ}$ lines in lifespan, bang sensitivity, and climbing assays and confirmed that these are strong loss-of-function alleles. In parallel, we generated human reference and variant UAS-cDNA lines of *PEX2* and *PEX16* variants in *Drosophila*. We observed nearly complete phenotypic rescue of *Drosophila Pex2* and *Pex16* loss when human $PEX2^{Ref}$ or $PEX16^{Ref}$, respectively, were expressed. We also provide evidence for an allele severity spectrum in *PEX2* and *PEX16* in which some missense alleles, such as $PEX2^{C247R}$, are equally severe as early truncations, such as $PEX2^{R119*}$. We also observed that alleles associated with mild PBD, such as $PEX2^{E55K}$, show variability depending on the assay but do not fully rescue. Finally, alleles associated with atypical ataxia phenotypes, such as $PEX16^{F332Del}$, can perform as well as $PEX16^{Ref}$, depending on the assay. Altogether, utilizing these unique *Drosophila* lines allows for effective disease modeling to understand the pathogenicity of peroxisomal biogenesis disorders and establish precise genotype-phenotype associations.

476F **The Impact of Dietary Interventions on Traumatic Brain Injury Responses in the Adult *Drosophila* CNS** Jesse J Rojas[1], Robert Squire[1], Andrew Kawwa[1], Kayla Lawani[1], Sydney Mudrak[1], Greenlee Kauinana[1], Marcus Intal[1], Kim D Finley[2] [1]Biology, San Diego State University, [2]San Diego State University

Millions of people worldwide sustain a wide range of head trauma that produce a diverse range of outcomes. Our previous research has highlighted the versatility of using *Drosophila* models to explore genetic and environmental factors that promote or erode the long-term function of the adult CNS (1,2). Aging and traumatic brain injury (**TBI**) based studies have identified autophagy pathway components (genetics) as well as dietary, probiotic and therapeutic treatments that improve neural function and promote healthy aging (1). In this report, we examine the potential role that dietary changes play on adult flies exposed to mild repetitive trauma (**mTBI**, 10x). Previous intermittent fasting (**IF**) studies showed improved neural health and autophagic function as adult flies age (2). From human studies, low carbohydrate, high fat ketogenic diets potentially showed neuroprotective promise after TBI exposure. Here, we examined whether IF exposure or high carbohydrate-caloric surplus similar to Western diets alters the trauma responses of adult flies (3-5). 10-days prior to trauma, male cohorts (+100) were started on IF treatment (1.5% agar, 8hrs, 3x weekly), maintained on standard *Drosophila* media (control) or food enriched with fructose or glucose (20%, w/v). After trauma exposure (Bead Ruptor method, 2.1 m/s, 10 bouts) each fly cohort was returned to its respective diet. Overall, we found that both IF treatment and high sugar diets, impacted key markers of acute and long-term trauma. This included global (mortality, longevity, inflammation profiles) as well as neuronal responses (climbing behaviors), while high sugar diets enhanced trauma related phenotypes. Neuroinflammatory marker levels (AMPs) are also being measured to examine the influence that diets may play on primary (4hr post mTBI) and secondary injury responses (1-day, 7-day post mTBI) and inflammation profiles. The goal of this study is to further characterize the close association noted between diet and basal inflammatory profiles with trauma related outcomes to highlight the potential effectiveness of non-invasive nutritional interventions for patients that have experienced head trauma.

477F **Evaluation of persistent environmental contaminants on conserved markers of survival and mechanisms associated with neural degeneration in *Drosophila*** Andrew Kawwa[1], Sara Laila[1], Jesse Rojas[2], Karilyn E Sant[3], Elana R Elkin[1], Kim D Finley[2] [1]School of Public Health, San Diego State University, [2]Biology, San Diego State University, [3]2College of Veterinary Medicine, Michigan State University

The incidence of neurodegenerative disorders is rapidly increasing, often times for unknown reasons. Several pervasive environmental contaminants such as trichloroethylene (TCE), tris(4-chlorophenyl)methane (TCPM), and their metabolites (DCVC-NAC, TCPMOH) are well known neurotoxicants. In turn, strong links between their environmental/occupational exposure and increased neurodegenerative disorder rates are ongoing areas of investigation. In addition, epidemiology studies clearly demonstrate people with protracted high-dose toxicant exposures are at higher risk for these disorders. However, the mechanism and long-term impact of low-dose toxicant exposures on neural function and maintenance is not well characterized. In this project, adult Drosophila were exposed to low doses of DCVC-NAC and TCPMOH and survival rates were measured. In addition, mRNA from fly neuronal tissues were isolated and RT-qPCR based studies used to evaluate select biological pathways implicated in neurodegeneration. For survival studies, one-week-old (adult) male flies were exposed to 0 (negative control) 10 or 20 µM DCVC-NAC throughout their lifespan. Additionally, one-week-old (adult) male or female flies were exposed to 0 (negative control), 0.1 µM, 1 µM, 5 µM, or 10 µM TCPMOH throughout their lifespan. The number of dead flies was recorded daily. Fly cohorts ingesting 20 µM DCVC-NAC had lifespan profiles decreased by 22.8% ($P<0.001$). Moreover, flies consuming food containing TCMPOH at concentrations as low as 1 µM in males and 5 µM in females also showed a significant reduction in adult longevity (~26%, $P<0.001$). Compared to untreated cohorts, a 24-hr TCPMOH (10 µM) exposure significantly increased *DptB and AttC* expression ($P<0.05$), indicating elevated inflammation. Our preliminary *Drosophila* results demonstrated that chronic, low dose exposure of several known environmental toxicants decreased survival rates and elevated neural stress markers. Additional examination of other neurodegeneration biomarkers expression patterns is ongoing. Our work seeks to shed light on the biological mechanisms underpinning associations between toxicant exposures and the development of progressive neurodegenerative disorders such as Parkinson's and Alzheimer›s disease.

478F **The tumor-induced host response** Hongcun Bao, Wu-Min Deng, Xianfeng Wang, Fei Cong Biochemistry & Molecular Biology, Tulane University

Cancer remains a major health concern, yet the mechanisms through which it leads to mortality are not fully understood. While studies of the mammalian immune system have produced transformative cancer therapies, the system's complexity poses challenges for further exploration. In contrast, the fruit fly (*Drosophila melanogaster*) offers a simpler model, providing unique advantages for studying tumor-host interactions. Here, we leverage this model's simplicity to investigate the mechanisms of tumor-induced lethality.

Using a Notch Intracellular Domain (NICD) overexpression tumor model, we developed an allograft system in which larval salivary gland tumors were implanted into the abdomens of adult flies. The tumors grew in the host for 10 days, after which they were dissected, fragmented, and retransplanted for sustained growth—maintainable for up to two years. Throughout this period, we collected host and tumor tissues for RNA sequencing and proteomic analyses.

As a result, RNA sequencing and proteomic analyses identified a robust immune response in the host, and immunostaining confirming significant activation of the immune deficiency (IMD) pathway in fat body cells. Our findings suggest that this immune response is essential for sustaining the host's survival, offering insights into immune mechanisms that may be a key in host-tumor dynamics.

479F **Altered nociception in a *Drosophila* larvae model of Neurofibromatosis type 1** Anneke Knauss[1], Seth Tomchik[1,2] [1]Neuroscience and Pharmacology, University of Iowa, [2]Pediatrics, University of Iowa

Neurofibromatosis type 1 (NF-1) is an inherited monogenetic disorder caused by loss of function mutations in a single gene, neurofibromin 1. This gene encodes a large protein called neurofibromin (Nf1), a known tumor suppressor. In humans, the disease is diagnosed early in development and is characterized by both the formation of tumors in the nervous system and neuronal dysfunction. One of the most common symptoms of NF-1 is chronic pain, suggesting that nociceptive function is altered. The fruit fly, *Drosophila melanogaster*, is a powerful model for studying this disease; in *Drosophila*, the Nf1 protein is ~60% homologous to the human protein at the amino acid level. In this well-established animal model, nf1 mutants display neuronal and behavioral phenotypes reminiscent of human symptoms. In *Drosophila* larvae, loss of Nf1 induces neuronal hyperexcitability and tactile hypersensitivity; however, the links to pain and the underlying mechanisms of this disease model are unknown.

Larvae exhibit sophisticated nocifensive behavioral responses to noxious stimuli, which are produced by a well-characterized neuronal circuit. Using this paradigm, we found that loss of Nf1 increases the intensity and persistence of nocifensive behaviors in response to noxious heat, without altering the threshold for induction. Interestingly, while the behavioral phenotypes seen in adult flies can be recapitulated with pan-neuronal Nf1 RNAi, neuronal knockdown of Nf1 does not affect nociceptive responses. Here we report that loss of Nf1 increases nocifensive behavior in response to noxious heat, without altering the threshold for induction. To further dissect this phenomenon, we are using the sophisticated genetic toolkit available in flies to test the developmental requirement for Nf1, its metabolic contributions, and the localization of Nf1 function.

480F **Terazosin partially rescues synuclein-related phenotypes in a Drosophila model of Parkinson's disease** Jaidan Marano[1], Elaine R Reynolds[2] [1]Biology, Lafayette College, [2]Biology and Neuroscience, Lafayette College

Although no formal clinical trials have been done, people who take terazosin or related drugs has been shown to have a reduced risk of Parkinson's disease (PD) diagnosis and slower progression of the disease (Cai et al 2019). Terazosin is used clinically as an adrenergic receptor antagonist, however more recently it has also been shown to enhance glycolysis and ATP production by binding to phosphoglycerate kinase 1 (PGK1) (Chen et al 2015). Increased glycolysis in PD neurons may help overcome mitochondrial deficits associated with the diesase. In addition, a PGK1 mutant in Drosophila has been shown to have many of the phenotypes associated with PD (Shimizu et al 2019). We wanted to directly test the efficacy of terazosin in the alpha-synuclein Drosophila model, hoping to link the protein processing problems with metabolic rescue. Flies containing a Ddc-Gal4 construct were crossed with UAS synuclein flies and the offspring were raised on 10 uM terazocin or regular food without the drug through development and adulthood. Fly lifespan and climbing ability were monitored and flies were dissected later in their lifespan to assess dopaminergic loss. Terazosin partially rescued climbing ability but had no impact on lifespan. The results of an analysis of dopaminergic viability in aged flies will also be presented. Our results confirm the importance of metabolism to Parkinson's disease phenotypes and may provide a means to a better understanding of the causal elements of the model.

481F **Investigating the role of *NLGN3* in autism spectrum disorder and sleep disruptions** Rebekah Townsley[1], Jonathan Andrews[1], Sharayu Jangam[1], Kristy Jay[2], Michael Wangler[1,3] [1]Molecular and Human Genetics, Baylor College of Medicine, [2]Center for Genomic Medicine, Harvard, [3]Texas Children's Hospital

Autism spectrum disorder is a common neurodevelopmental disorder that is highly comorbid with sleep disruptions. Although 40-80% of individuals with ASD display sleep disruptions, this association is still largely unclear. Recently, variants in the *Neuroligin 3* (*NLGN3*) gene have been identified in patients with both ASD and sleep disorders. NLGN3 is a postsynaptic cell adhesion molecule that interacts with neurexins to regulate synapse formation and maturation. Previous studies have indicated that loss-of-function (LoF) *NLGN3* mutants exhibit primary phenotypes of ASD and secondary phenotypes of sleep disruption suggesting that disruption in this gene may contribute to both disorders; however, the driving mechanism is still unknown. To address this gap, we have prioritized three *NLGN3* variants (p.R175W, p.R451C, and p.R597W) for functional analysis in *Drosophila*. All three variants were identified in individuals with ASD and the latter variant is also associated with sleep disorders. We identified that the loss of *Nlg3*, the *Drosophila* ortholog, led to a reduction in locomotor activity under a light/dark cycle. Furthermore, these same mutant flies also displayed abnormal sleep consolidation. Using the overexpression system, we showed that overexpressing human *NLGN3*-Ref and the *NLGN3*-R597W variant causes abnormal sleep consolidation. To provide a mechanistic understanding for these behavioral changes, we turned to the neuromuscular junction (NMJ). We found that *Nlg3* negatively regulates bouton growth at the NMJ and plays an important role in regulating mEJP. Using rescue paradigms, we were able to rescue LoF phenotypes when either wildtype *Nlg3* or human *NLGN3* was expressed in a null background. Additionally, our preliminary data suggests that the *NLGN3*-R175W and *NLGN3*-R451C variants are unable to rescue these bouton phenotypes, while the *NLGN3*-R597W variant can partially rescue. These early results indicate that the *NLGN3*-R175W variant is acting in a loss-of-function mechanism whereas the *NLGN3*-R597W variant may be a gain-of-function. Taken together, our work demonstrates that human *NLGN3* can restore normal function in *Drosophila* and alterations in this gene can impair sleep. Furthermore, we have developed a pipeline to assess ASD-associated *NLGN3* variants in *Drosophila*. Our goal is to provide a functional link between ASD and sleep disturbances to identify potential therapeutic approaches to lessen the impact of sleep abnormalities in individuals with ASD.

482F **Toward a molecular understanding of tissue-specific phenotypes in RNA exosome-linked neurodevelopmental disorders** Lauryn A Higginson, Derrick J Morton Biological Sciences, University of Southern California

Post-transcriptional regulation of gene expression is critical for proper neuronal development and function. Many of these highly coordinated post-transcriptional regulatory events are mediated by an evolutionarily conserved and ubiquitously expressed RNA processing complex, the RNA exosome. Recent clinical reports have linked autosomal recessive missense mutations in the genes encoding structural subunits of the RNA exosome to distinct tissue-specific disorders with shared neurological features. Disease-linked mutations in RNA exosome subunit genes: *EXOSC3* and *EXOSC9* cause distinct subtypes of a devastating neurodevelopmental disorder, Pontocerebellar Hypoplasia. In contrast, mutations in RNA exosome subunit gene *EXOSC2* cause a novel syndrome, SHRF (**S**hort stature, **H**earing loss, **R**etinitis pigmentosa, and distinctive **F**acies), with mild cerebellar atrophy. These observations indicate tissue-specific function for the RNA exosome and an enhanced requirement for the complex in neurodevelopment. Towards understanding the biological mechanism of RNA exosome-linked neuronal dysfunction, my studies will focus on systematically investigating and comparing the cell type/tissue-specificity of pathogenic variants in distinct RNA exosome subunits in the fly brain. Thus, I have engineered flies modeling pathogenic mutations in RNA exosome Cap subunit genes: EXOSC2 (fly Rrp4), EXOSC3 (fly Rrp40), and Core subunit gene EXOSC9 (fly Rrp45) via CRISPR/Cas9. My preliminary studies show that distinct pathogenic variants within different or the same RNA exosome gene cause a spectrum of organismal phenotypes including reduced viability, behavioral defects, and defects in brain morphology compared to wildtype control flies. Furthermore, to examine RNA exosome complex levels/integrity, we utilized mass-spectrometry based approaches in brain-enriched tissue of RNA exosome mutant flies compared to wildtype controls. In sum, this study advances our understanding of RNA exosome-linked neurological disease and provides insight into the distinct tissue-specific consequences caused by alterations in subunits within a single RNA processing complex.

483F **Phagocytic glia mediate protein aggregate propagation in neurodegenerative diseases** Graham H. Davis[1], Kirby M. Donnelly[2], Kathleen Wooster[3], Margaret M. Panning Pearce[1] [1]Department of Biological & Biomedical Sciences, College of Science and Mathematics, Rowan University, [2]Department of Biology, St. Joseph's University, [3]College of Science and Mathematics, Rowan University

Neurodegenerative diseases such as Alzheimer's Disease (AD) and Huntington's Disease (HD) are increasing in prevalence in our aging population and currently have no cure. All neurodegenerative diseases are characterized by progressive loss of neurons as well as glial cell impairment caused by an accumulation of neurotoxic protein aggregates in vulnerable brain regions. Accumulating evidence supports the hypothesis that pathological proteins such as mutant huntingtin (mHTT) in HD and microtubule-associated protein Tau in AD and related tauopathies possess prion-like behaviors–they spread from cell to cell via templated aggregation of natively-folded proteins. Increasing our understanding of the mechanisms that underlie prion-like propagation is essential to identify novel targets for improved therapeutic interventions.

Our lab has reported that mHTT aggregates undergo prion-like transfer between neurons and glia in adult *Drosophila* brains via the well-conserved glial scavenger receptor, Draper/MEGF10. *Draper-I* transcript levels increased in response to mHTT expression in neurons, and mHTT aggregate numbers were elevated in *draper*-deficient flies, suggesting that Draper-dependent phagocytosis regulates the burden of pathogenic aggregates in the brain. Remarkably, mHTT aggregates formed in presynaptic olfactory receptor neurons (ORNs) seeded the aggregation of normally-soluble wild-type huntingtin (wtHTT) proteins in ensheathing glial cells, followed by postsynaptic projection neurons (PNs). These findings indicate that Draper+ phagocytic glia serve as a conduit for prion-like spread of mHTT aggregates across synapses. Our current efforts explore mechanisms regulating pathological tau burden and spreading in adult fly brains, including examining roles for Draper/MEGF10 and downstream phagocytic pathway components. Defects in multiple phagocytic pathway steps, including recognition, engulfment, and phagolysosomal degradation, are implicated in the accumulation of diverse protein aggregates, suggesting that phagocytic glia may play a central role in the progression of protein pathology in all neurodegenerative disorders.

484F ***Drosophila* Models for Charcot-Marie-Tooth Neuropathy Related to Methionyl-tRNA Synthetase.** jung jieun Disease Target Structure Research Center, Korea Research Institute of Bioscience & Biotechnology

***Drosophila* Models for Charcot-Marie-Tooth Neuropathy Related to Methionyl-tRNA Synthetase.**

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Charcot-Marie-Tooth disease (CMT) is one of the most prevalent inherited peripheral neuropathies. Patients with CMT typically experience atrophy of the peripheral muscles, resulting in abnormal changes in the shape of their hands and feet. CMT can be broadly categorized into demyelinating Charcot-Marie-Tooth disease (CMT1) and axonal degenerative Charcot-Marie-Tooth disease (CMT2). CMT2 is associated with more than 30 loci, and approximately 20 causal genes have been identified.

Aminoacyl-tRNA synthetase (aaRS) is an enzyme responsible for attaching specific amino acids to tRNA in an ATP-dependent manner. To date, six types of aaRS mutations have been identified as the underlying cause of autosomal dominant axonal or intermediate Charcot-Marie-Tooth (CMT) disease: glycyl-tRNA synthetase (GARS), tyrosyl-tRNA synthetase (YARS), alanyl-tRNA synthetase (AARS), histidyl-tRNA synthetase (HARS), lysyl-tRNA synthetase (KARS), and methionyl-tRNA synthetase (MARS). In this study, we present a *Drosophila* model for Charcot-Marie-Tooth disease (CMT) featuring mutations in methionyl-tRNA synthetase (MARS). The findings are based on the phenotypic analysis of the CMT model using four MARS mutants, which include previously reported mutant lines (R618C, P800T) as well as newly introduced mutant lines (E86R, A393T).

485F **The Role of PINK1 Modulation in Alzheimer's Disease Progression and Mitochondrial Health in *Drosophila melanogaster*** Luciane C Alberici[1], Giulia C Spegiorim[2] [1]University of Sao Paulo, [2]Biochemistry, University of Sao Paulo

Alzheimer's disease (AD) is a progressive neurodegenerative disorder, mainly defined by the loss of memory and cognitive function. The key pathological features include intracellular neurofibrillary tangles and extracellular beta-amyloid plaques, which are known for their neurotoxic effects. As the disease progresses, these features are accompanied by inflammation, oxidative stress, and ultimately lead to neuronal death. Mitochondrial and mitophagy dysfunction, characterized by reduced ATP production, elevated oxidative stress and reduced mitochondrial renovation, has emerged as a significant marker in AD and may even precede the formation of beta-amyloid aggregates and neurofibrillary tangles. This study aimed to explore the connection between the mitophagy pathway and AD progression. Using the UAS-Gal4 expression system, we performed both positive and negative modulation of the PINK1 gene expression in two *Drosophila melanogaster* models for AD – one expressing the human proteins APP and BACE pan-neurally, and the other expressing only the Aβ42 peptide with the *Arctic* mutation. Behavioral phenotypes, such as survival, climbing (locomotion), and memory were evaluated, as well as the functional characterization of mitochondria in the central nervous system through the assessment of oxygen consumption and the quantification of reactive oxygen species (ROS) production. Our findings revealed that in the APP-BACE model, PINK1 overexpression extended lifespan and partially rescued memory deficits but did not enhance locomotor performance. Although O_2 consumption remained unchanged in the heads of these flies, PINK1 overexpression significantly reduced elevated ROS levels. Knockdown of PINK1 via RNAi in the same model exacerbated the decline in survival, with no substantial changes in the other phenotypes. In the Aβ42 model, both upregulation and downregulation of PINK1 improved survival, while PINK1 RNAi reversed the observed reductions in O_2 consumption and increased ROS production. PINK1 overexpression had little effect on the other phenotypes beyond survival. In summary, these results suggest that enhancing PINK1/Parkin-mediated mitophagy can prolong survival in AD models. However, it is insufficient to fully restore the phenotype, pointing to the complex and multifaceted role of mitochondrial function in the systemic pathology of AD.

486F **Inhibition of the MEK/ERK pathway suppresses immune overactivation and mitigates TDP-43 toxicity in a *Drosophila* model of ALS** Wenkai Yue, Xue Deng Interdisciplinary Research Center on Biology and Chemistry, Chinese Academy of Sciences

TDP-43 is an important DNA/RNA-binding protein that is associated with age-related neurodegenerative diseases such as amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD); however, its pathomechanism is not fully understood. In a transgenic RNAi screen using *Drosophila* as a model, we uncovered that knockdown (KD) of *Dsor1* (the *Drosophila* MAPK kinase *dMEK*) suppressed TDP-43 toxicity without altering TDP-43 phosphorylation or protein levels. Further investigation revealed that the *Dsor1* downstream gene *rl* (*dERK*) was abnormally upregulated in TDP-43 flies, and neuronal overexpression of *dERK* induced profound upregulation of antimicrobial peptides (AMPs). We also detected a robust immune overactivation in TDP-43 flies, which could be suppressed by downregulation of the MEK/ERK pathway in TDP-43 fly neurons. Furthermore, neuronal KD of abnormally increased AMPs improved the motor function of TDP-43 flies. On the other hand, neuronal KD of *Dnr1*, a negative regulator of the *Drosophila* immune deficiency (IMD) pathway, activated the innate immunity and boosted AMP expression independent of the regulation by the MEK/ERK pathway, which diminished the mitigating effect of RNAi-*dMEK* on TDP-43 toxicity. Finally, we showed that an FDA-approved MEK inhibitor trametinib markedly suppressed immune overactivation, alleviated motor deficits and prolonged the lifespan of TDP-43 flies, but did not exhibit a lifespan-extending effect in Alzheimer disease (AD) or spinocerebellar ataxia type 3 (SCA3) fly models. Together, our findings suggest an important role of abnormal elevation of the MEK/ERK signaling and innate immunity in TDP-43 pathogenesis and propose trametinib as a potential therapeutic agent for ALS and other TDP-43-related diseases.

487F **Malpighian tubule phenotypes in the *park^{25}* model of Parkinson's disease** Samantha M. Chagolla[1,2], Krista Pearman[3], Gerald B. Call[1,3] [1]College of Dental Medicine Arizona, Midwestern University, [2]Biomedical Sciences Program, College of Graduate Studies, Midwestern University, [3]Department of Pharmacology, College of Graduate Studies, Midwestern University

The *Drosophila melanogaster parkin* (*park*) gene is the ortholog to the human *PRKN* gene, which when mutated in humans leads to a genetic form of Parkinson's disease (PD). As such, homozygous *park^{25} Drosophila* is a frequently used model to study PD. These flies display phenotypes similar to the hallmark symptoms of PD that include selective loss of dopaminergic neurons, impaired motor function, loss of olfaction, decreased lifespan, and mitochondrial dysfunction. However, while previous studies have given valuable information on *park*^{25} flies and their symptoms, our recent research has revealed an unexpected background mutation in our *park*^{25} flies. Our initial observations showed the presence of stones in enlarged Malpighian tubules (MTs) in homozygous *park^{25}* flies. SEM and electron microprobe analysis revealed that the stones are mostly composed of an organic material with the presence of calcium phosphate nodules. Based on our findings, the *park*^{25} flies have stones present as early as the pupae stage and have an increased stone burden with age, from day 0 to 15 and 20. It was observed that the location of the stones was mostly only in the posterior pair of MTs (69%) in comparison to only 1% in the anterior pair. It is also important to note that, just like the increasing burden of stones that was see with age, it was also observed that the size of the MTs also increased with age. However, after transheterozygous and other genetic analysis and genomic sequencing we were able to identify the presence of a novel mutant allele in the *rosy* (*ry*) gene. The *ry* gene encodes the xanthine dehydrogenase enzyme and its mutation is well known for the production and accumulation of xanthine stones in the MTs. It was determined that stones are present in a *park^{25}*/*ry^{1}* cross, identifying that the *ry* mutation is the cause of the MT stones in *park*^{25} flies. However, this cross did not have as high a percentage of stones present as the *park^{25}* homozygotes. In addition, *park* transheterozygotes still had enlarged MTs compared to controls. These findings indicate that *park* leads to MT phenotypes and possibly synergistically interacts with *ry* for the production of MT stones.

488F **Use of *Drosophila melanogaster* to Investigate Pharmacotherapies for Alcohol Use Disorder** Rebecca Oramas[1], Yanabah Jacques[1,2], Natalie D'Silva[1], Reza Azanchi[1], John E McGeary[3], Karla Kaun[1] [1]Neuroscience, Brown University, [2]Neuroscience, University of California Berkeley, [3]Psychiatry & Human Behavior, Brown University

Drosophila melanogaster has been an effective *in vivo* model for therapeutic discovery of central nervous system disorders, inflammatory disorders, cardiovascular disease, cancer, and diabetes. The ability to perform high-throughput drug studies in such a well-studied model organism provides a powerful whole-organism approach to identifying potential pharmacotherapies. There are currently very few studies investigating the use of *Drosophila* for investigation and discovery of pharmacological targets of Alcohol Use Disorder (AUD). Several drugs have been developed to treat AUDs, including Naltrexone and Acamprosate, and others prescribed off-label such as Topiramate. Although the effects of these drugs are well-studied, their mechanisms remain obscure. To validate *Drosophila* as an effective model for studying pharmacological therapies for AUD, we identified non-disruptive drug doses in flies and then assessed their impact on ethanol consumption and conditioned preference in cue-induced alcohol-seeking after 1 or 3 days of training. Naltrexone and Acamprosate significantly reduced ethanol food preference and conditioned preferences after 3 days, while Topiramate did not. Additionally, we explored the effects of a novel class of drugs for the treatment of AUD, by investigating how inhibiting Notch signaling with gamma-secretase inhibitors Dibenzazepine and Compound E impact alcohol preference. We found that Dibenzazepine effectively reduced ethanol consumption, and both inhibitors decreased conditioned preference after 3 days. These results suggest that *Drosophila* is a valuable platform for identifying and characterizing new AUD treatments.

489F **Human muscular dystrophy caused by JAG2 (Serrate in Drosophila) mutations** Nam Chul Kim[1], Isabelle Draper[2], Atsushi Asakura[3], Peter Kang[3] [1]Pharmacy Practice and Pharmaceutical Sciences, University of Minnesota, [2]University of Minnesota, [3]Neurology, University of Minnesota

We recently identified homozygous and compound heterozygous mutations in JAG2 (Serrate in Drosophila), a ligand for Notch receptor 1. Nevertheless, the precise manner by which mutations in the JAG2 gene affect muscle stem cell function and muscle regeneration remained unclear. Accordingly, we sought to understand the ramifications of Jag2 malfunction within the muscle stem cell niche, employing a range of model systems. The knockdown of Serrate with Ser-GAL4 in Drosophila resulted in significant developmental abnormalities in the adult stage, including motor defects and a shortened lifespan. Additionally, it resulted in the formation of pronounced melanotic/necrotic tissue in the leg joints. The wild-type JAG2, but not the mutant JAG2 variants, was capable of rescuing the deficiency of the Ser gene. Co-culture experiments and conditional Jag2 mutant mice demonstrated that endothelial cell-specific Jag2 deletion reduces satellite cell self-renewal, whereas satellite cell-specific Jag2 deletion reduces myogenic differentiation. Therefore, mutations in Jag2 result in aberrant muscle regeneration through both trans- and cis-activation, and contribute to muscular dystrophy through impaired Notch signaling. Our study provides evidence for a muscular dystrophy disease mechanism involving dysfunction of the Notch pathway.

490F **Cachexia triggered by microbiota-host interaction** Yuya Sanaki[1], Carine Ganem-Elbaz[2], Bhavya Gummadi[1], Akira Goto[3], Di Chen[3], Chih-Chiang Chan[4], François Leulier[5], Ryusuke Niwa[1], Pierre Leopold[2] [1]Life Science Center for Survival Dynamics, TARA, University of Tsukuba, [2]Institut Curie, Paris – INSERM,Institut Curie -PSL Research University, [3]Insect Models of Innate Immunity (M3I; UPR9022) – University of Strasbourg– CNRS, [4]"Graduate Institute of Physiology, National Taiwan University, [5]IGFL, ENS Lyon, CNRS, Université Claude Bernard Lyon 1

Cachexia is a complex metabolic disorder characterized by progressive organ wasting that limits the survival of tumor-bearing patients. While previous studies revealed important roles of inflammation and hormonal imbalance in cachectic progression, the fundamental risk factors contributing to cachexia onset remain elusive. Using Drosophila as a model for tumor-induced cachexia, we report the systemic role of gut microbiota in tumor-induced host metabolic decline. Adult flies injected with tumor cells and reared in control conditions showed significant wasting of muscle and adipose tissues starting at moderate tumor load. Alternately, antibiotic treatment eliminating microbiota prevented cachexia progression even at a large tumor load. These findings suggest that the gut microbiota influences "tumor load tolerance" referred to as the ability of the host to cope with developing tumors without cachexia. Mechanistically, tumor-bearing animals showed gut microbiota dysbiosis with an overabundance of *Escherichia coli*. We found that non-pathogenic *E. coli* is sufficient to trigger cachexia through activation of its aerobic metabolism, while the responsible metabolites are still unknown. Moreover, multiomics and RNAi screening in the fly host revealed that microbiota promotes the transcriptional activation of specific metabolic pathways in the midgut R4 and is associated with systemic organ wasting. In conclusion, we find that gut microbiota metabolite(s) from aerobic respiration influence(s) gene expression profile in the host gut, restricting tumor load tolerance and promoting cachexia onset, thereby limiting the survival of tumor-bearing flies.

491F ***Drosophila* ‹kidney-gut› communication: insights from the single-nucleus RNA-sequencing** Jun Xu[1], Norbert Perrimon[2] [1]CAS Center for Excellence in Molecular Plant Science, [2]Genetics, Harvard Medical School

Paraneoplastic syndromes occur in cancer patients and originate from dysfunction of organs at a distance from the tumor or its metastasis. A wide range of organs can be affected in paraneoplastic syndromes; however, the pathological mechanisms by which tumors influence host organs are poorly understood. Recent studies in the fly uncovered that tumor secreted factors target host organs, leading to pathological effects. In this study, using a Drosophila gut tumor model, we characterize a mechanism of tumor-induced kidney dysfunction. Specifically, we find that Pvf1, a PDGF/VEGF signaling ligand, secreted by gut tumors activates the PvR/JNK/Jra signaling pathway in the principal cells of the kidney, leading to mis-expression of renal genes and paraneoplastic renal syndrome-like phenotypes. Our study describes an important mechanism by which gut tumors perturb the function of the kidney, which might be of clinical relevance for the treatment of paraneoplastic syndromes.

492F **Sexually dimorphic neurodevelopment, neural activity, behavior and gene expression in Chd1altered Drosophila** Sadam Hussain[1], Safa Salim[1], Ayesha Banu[1], Mohammad Farhan[2] [1]Biological & Biomedical Sciences, College of Health & Life Sciences, [2]Biopscychology & Neuroscience, College of Health & Life Sciences

<p break-words”=””>Chromodomain helicase DNA binding proteins (CHD1 and CHD2) are essential epigenetic regulators, yet their sex-specific roles in neurodevelopment remain poorly understood. Here, we investigate the dimorphic functions of CHD1 and CHD2 orthologs in Drosophila neural development and behavior. Using behavioral assays combined with gene expression analysis, we demonstrate that these chromatin remodelers exhibit sex-specific regulation of sleep architecture and social interactions. Our findings reveal distinct patterns of CHD1 and CHD2 activity in male versus female flies, particularly in modulating neural circuit development and activity patterns. This sexual dimorphism in CHD-mediated chromatin regulation provides crucial insights into the epigenetic basis of sex-specific neurodevelopment. These results have important implications for understanding sex-biased prevalence in human neurodevelopmental disorders and sleep disturbances, potentially opening new avenues for sex-specific therapeutic interventions.

<p break-words”=””>Keywords: CHD1, CHD2, chromatin remodeling, sexual dimorphism, neurodevelopment, Drosophila, sleep behavior

493F **Adipose and Cardiac Fatty Acid Transport Protein 1 (FatP1)-mediated Lipid Uptake is required to Maintain Cardiac Function** Giuseppe Trimarchi[1], Melanie Grieger[2], Georg Vogler[3], Rolf Bodmer[3], Stephan Sigrist[2], Anna Foryst-Ludwig[1], Ulrich Kintscher[1] [1]Institute of Pharmacology, Charité Universitätsmedizin, [2]Institute of Biology, Freie Universität Berlin, [3]Center for Genetic Disorders and Aging Research, Sanford Burnham Prebys Medical Discovery Institute

Heart failure is an age-related disease characterized by systolic and diastolic dysfunction. Previous studies have indicated that modifications in lipid metabolism can delay the onset of heart failure. In our study we investigate the role of lipid transport in the development of cardiac dysfunction by generating tissue-specific knockdowns of the fatty acid (FA) transport protein 1 (FatP1) in both cardiac and adipose (fat body) tissues in Drosophila melanogaster.

We generated Drosophila melanogaster models with heart-specific (+/+; Hand4.2-GAL4/UAS-FatP1; tdtK/+) (cFatP1-KD) and fat body-specific (+/+; ppl-GAL4/UAS-FatP1; tdtK/+) (atFatP1-KD) knockdowns of FatP1 using the UAS/GAL4 system. Cardiomyocytes in these flies endogenously express tdTomato, enabling live cardiac imaging through fluorescence-based high-resolution video microscopy. Cardiac morphology and lipid accumulation were evaluated using confocal microscopy. Further, mitochondrial function was assessed via glycolytic Seahorse assay, as well as with TMRM, Amplex Red, and luciferase assays on isolated hearts.

In atFatP1-KD flies, inhibition of fatty acid (FA) uptake in adipose tissue resulted in a reduction in relaxation velocity ($p=0.0009$, WT vs. atFatP1-KD), a marker of diastolic dysfunction, while contraction velocity and fractional shortening (systolic function) were unaffected. This dysfunction was accompanied by an increase in the number and size of cardiac lipid droplets (LDs) ($p=0.0275$ and $p<0.0001$, WT vs. atFatP1-KD). In addition, mitochondrial function in the heart was compromised in atFatP1-KD flies ($p=0.0307$, WT vs. atFatP1-KD). ROS levels and ATP content within the heart were elevated ($p=0.014$ and $p=0.0342$, WT vs. atFatP1-KD).

In heart-specific FatP1 KD (cFatP1-KD) flies, reduced cardiac FA uptake led to a decrease in end-diastolic diameter and a substantial reduction in both relaxation and contraction velocities ($p<0.0001$ and $p=0.0001$, WT vs. cFatP1-KD), indicating impairments in both diastolic and systolic function. The number of LDs is reduced as expected ($p=0.0625$, WT vs. cFatP1-KD). Although ROS and ATP levels within the heart tube were elevated ($p=0.005$ and $p=0.0032$, WT vs. cFatP1-KD), mitochondrial function remained unaffected.

This study supports the hypothesis that reduced FA uptake in adipose tissue results in elevated cardiac lipid levels, triggering mitochondrial dysfunction and subsequent cardiac remodeling leading to diastolic dysfunction. Interestingly, limiting cardiac lipid droplet uptake induces systolic and diastolic cardiac dysfunction in Drosophila melanogaster driven by ROS formation independent of mitochondrial pathways, suggesting an alternative mechanism. In summary, these findings highlight the importance of a functional cardiac–adipose tissue axis which requires intact FatP1-mediated lipid uptake in both organs to maintain cardiac function

494F **MEK inhibition as a potential therapeutic strategy for the non-tumor manifestations of neurofibromatosis type 1 (NF1)** Alex Dyson[1], Jadwiga N Bilchak[2], Genesis Omana Suarez[3], Nicholas C. LoRocco[1], Torrey Mandigo[1], Jahanbanoo Shahryari[4], Scott R Plotkin[1], Seth Tomchik[3], Matthew Kayser[2], James Walker[1] [1]Massachusetts General Hospital, [2]University of Pennsylvania, [3]University of Iowa, [4]NFlection Therapeutics

Neurofibromatosis type 1 (NF1) is a neurodevelopmental condition arising from loss-of-function mutations in the *NF1* gene on chromosome 17. Whilst commonly classified as a tumor-predisposition syndrome, up to 80% of affected individuals also display some form of behavioral and/or cognitive impairment, including specific learning difficulties, sleep disturbances, and autism spectrum disorder. Although the MAPK/ERK kinase (MEK) inhibitor selumetinib was recently approved for the treatment of inoperable plexiform neurofibromas in NF1, there are currently no treatments available for the aforementioned neurological symptoms of the disorder. Whether MEK inhibition also provides a suitable therapeutic strategy for these is unclear.

The *Drosophila* genome contains a highly conserved ortholog of human *NF1* (*dNf1*), knockout of which has been shown to give rise to numerous phenotypes reminiscent of the clinical condition. Thus, fly models of NF1 provide valuable systems with which to investigate potential therapeutic targets for the disorder. Here, we use CRISPR technology to generate novel *dNf1* mutant alleles that recapitulate established $dNf1^{-/-}$ phenotypes, including reduced growth, cognitive deficits, tactile hyper-responsiveness, excessive grooming, circadian rhythm disruption, and impaired courtship. Pan-neuronal knockdown of MEK via RNA interference rescues these defects, supporting MEK inhibition as a potential therapeutic strategy for the behavioral symptoms of NF1 in the clinic. Efforts to determine whether pharmacological (as opposed to genetic) inhibition of MEK can also improve $dNf1^{-/-}$ behavioral phenotypes are currently ongoing.

495F **FUS aggregates lead to synaptic microtubule disruption in an ALS model** Tulika Malik, Sam Jones, Daniel T Babcock Biological sciences, Lehigh University

Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disorder that primarily affects motor neurons in the brain and spinal cord. ALS patients usually have an average life expectancy of 2-5 years after diagnosis. Synaptic defects prior to neural loss is one of the earliest hallmarks of neurodegenerative disorder. Therefore, targeting early synaptic defects is crucial to understanding neurodegenerative disorders like ALS. To better understand synaptic defects in ALS, we expressed either wild-type or mutant Fused in Sarcoma (FUS) in adult motor neurons. FUS is an RNA binding protein that is often mislocalized in a particularly aggressive form of ALS. There has been evidence of FUS aggregates forming in the cytoplasm, but how these aggregates lead to synaptic and cytoskeleton defects is unknown. Our preliminary evidence indicates that overexpression of FUS leads to loss and fragmentation of Futsch (MAP1B). We observe FUS aggregates accumulating at synaptic terminals in adult abdominal NMJs. Our goal is to study the mechanism behind cytoskeleton destabilization and MAP1B fragmentation in a FUS-mediated ALS model in motor neurons in adult Drosophila. Additionally, we uncover co-localization FUS aggregates and Futsch at the adult NMJ. We hypothesize that synaptic defects occur due to FUS aggregates interacting with different microtubule-associated proteins such as Futsch, APC, and EB1. Could preventing microtubule disruption or preventing aggregate formation be neuroprotective against ALS? This work suggests how FUS aggregates play a major role in synaptic pathology and a potential target for the FUS-ALS model.

496F **A drug repurposing screen identifies NSIADs and COX1/2 enzyme inhibition as potential therapies for MAN1B1-CDG, a rare congenital disorder of glycosylation.** Katherine Beebe[1], Kevin A Hope[1], Emily Coelho[1], Heather D Evans[1], Caroline Massey[1], Claire Fast[2], Ethan O Perlstein[3], Clement Y. Chow[1] [1]University of Utah, [2]University of British Columbia, [3]Perlara

MAN1B1-CDG is a congenital disorder of glycosylation caused by autosomal recessive mutations in the MAN1B1 gene. MAN1B1-CDG is characterized by intellectual and developmental delay, hypotonia, truncal obesity, verbal and physical aggression, and facial dysmorphisms. Clinical symptoms common to other CDGs, like seizures are rare. MAN1-B1-CDG is a rare disorder with fewer than 100 patients reported. The MAN1B1 gene encodes a protein localized to the endoplasmic reticulum and Golgi and plays several different roles in protein quality control. Like most rare disorders, MAN1B1 CDG lacks any therapeutic treatments. Most treatments are focused on the management of symptoms. Drug repurposing provides a rapid way forward for identify drugs that might treat rare diseases. Drug repurposing involves the reuse of FDA approved drugs in new applications. In particular, there are likely approve drugs that may be applied to rare diseases. This approach bypasses the years or even decades of drug development needed to bring a new molecule to the clinic. To find new potential treatments for MAN1B1-CDG, we performed a drug repurposing screen in Drosophila for MAN1B1-CDG. We developed a rough-eye model of MAN1B1 loss of function and subjected the model to 1520 FDA approved drugs. We found 50 drugs that provided rescue and 47 drugs that enhanced or worsened the eye phenotype. Nearly 20% of the drugs that provided rescue were non-steroidal anti-inflammatory drugs (NSAIDs). Nearly all the NSAIDs showed rescue in an independent dose curve validation experiment. Because NSAIDs primarily block COX1/2 enzyme activity, we tested whether genetic reduction of COX enzyme activity could mimic NSAID rescue. RNAi knockdown of *Drosophila* COX enzyme provided strong rescue of the MAN1B1-CDG eye model. Finally, we tested the strongest NSAID from the screen, Ibuprofen, on whether it could rescue nervous system dysfunction associated with loss of MAN1B1 in *Drosophila*. Ibuprofen was able to provide strong rescue of seizure behavior in a neuron-specific knockdown of MAN1B1. An N=1 study with ibuprofen is currently underway in a child living with MAN1B1-CDG. Together, this study indicates that inhibition of COX activity by NSAIDs might be a viable therapeutic approach for MAN1B1-CDG.

497F **The interactome of the microcephaly-associated protein Abnormal Spindle reveals a role for protein phosphatase 2A in regulating brain growth and development** Steven J Florez[1], Todd Schoborg[2] [1]molecular biology, University of Wyoming, [2]University of Wyoming

Mutations in *abnormal spindle (asp)* disrupt brain growth and development, yet the underlying mechanisms responsible have yet to be defined. This is primarily due to a lack of known protein interactors, which can provide insight into the cellular pathways essential for this process. Here we report the identification of >20 novel interactors of Asp's N-terminus, which we previously showed to be essential for brain growth and development. Using a genome-wide Yeast two-hybrid (Y2H) fragment library from the third instar larval brain, we obtained domain level resolution of these interactions. The highest confidence partners were the β-regulatory subunits of Protein Phosphatase 2A (PP2A), including widerborst (wdb) and well-rounded (wrd). AlphaFold 3 modeling confirmed that this interaction is driven through a short linear motif (SLiM, LACIHEEE) in Asp's unstructured region that becomes stronger upon phosphorylation of nearby S/T residues. Mutations in Asp's SLiM motif led to a reduction in fly brain size and disrupted morphology, particularly in the inner proliferation center (IPC) of third instar larva and the lobula, lobula plate, and mushroom bodies of adult flies. AlphaFold3 modeling of these Asp SLiM mutants confirmed that only the interactions with wdb and wrd were disrupted. These results suggest that the Asp/PP2A interaction is critical for brain growth and development.

498F **Evaluating the Effect of Nicotine Exposure on *Drosophila melanogaster*, a Model for Respiratory Diseases** Rene Toribio, Luis Trejo, Michael Zepeda, Nicole Bournias-Vardiabasis Biology, California State University, San Bernardino

Nicotine consumption through vaping has seen a concerning rise in recent years, particularly prevalent among high school students despite declines in cigarette use. In 2023, 10.0% of high school students reported regular e-cigarette use, according to the FDA's National Youth Tobacco Survey (CDC). Numerous studies highlight the negative side effects of nicotine, such as COPD, respiratory disease, heart disease, and early death among consumers. To evaluate the effects of vape products on respiratory health, we investigated how vaping impacted the trachea of *Drosophila melanogaster* 3rd instar larvae. *Drosophila* is a well-established model organism due to its genetic tractability, short life cycle, and similarities in fundamental biological processes to humans. The larvae provide a useful model for studying respiratory diseases due to structural and functional similarities with mammalian respiratory systems. The *Drosophila* tracheal system has been widely utilized to uncover mechanisms behind tube morphogenesis, which can be linked to structural changes observed in COPD, making it a valuable tool for studying COPD-related alterations. We conducted assays to assess nicotine-laced food effects on the viability of *Drosophila* 3rd instar larvae, using this data to determine appropriate nicotine concentrations for vaping exposure. The effects on viability, development, and respiratory damage were assessed, with ingestion and inhalation resulting in a substantial, dose-dependent decrease in larval survival. Dissections and histological analysis of the trachea revealed significant morphological differences, indicating structural damage. Additionally, we utilized flow cytometry to further analyze cellular changes in tracheal tissue after exposure, providing detailed insights into the impact of nicotine on respiratory cell populations. These studies demonstrate *Drosophila melanogaster* as a viable model for investigating the impact of nicotine ingestion and inhalation, revealing both macroscopic and cellular-level alterations associated with respiratory damage, and contributing to a deeper understanding of the health risks posed by e-cigarette use.

499F **Nf1 regulates metabolism via developmental effects** Catherine Steele, Seth Tomchik Neuroscience and Pharmacology, University of Iowa

Neurofibromatosis type 1 is a genetic disorder that predisposes individuals to tumors and neurocognitive symptoms. In addition, individuals with neurofibromatosis type 1 may have altered body composition, increased insulin sensitivity, and greater fatigue, suggesting metabolic alterations. Further, neurofibromatosis type 1 patients exhibit alterations in lipid utilization and resting energy expenditure (particularly in females). The mechanisms underlying these effects are not well understood. Neurofibromatosis type 1 is caused by loss-of-function mutations in a single gene, neurofibromin 1. This gene encodes a large protein, called neurofibromin (Nf1), which regulates multiple cellular and molecular pathways. Nf1 has a central GAP-related domain that functions to inhibit Ras signaling. Nf1 deficiency therefore increases Ras activation, along with its downstream effectors.

Nf1 modulates metabolism in Drosophila. Flies express an Nf1 ortholog that is ~60% identical to the human Nf1 protein and shares conserved Ras GAP activity. Previous studies have reported that Nf1 deficiency modulates multiple metabolic parameters in *Drosophila*. Specifically, loss of Nf1 increases CO_2 production and O_2 consumption, and reduces the respiratory quotient, suggesting increased utilization of fat as an energy source. Consistent with that prediction, Nf1 deficiency decreases energy stores, reducing triglyceride levels and starvation survival. Feeding is increased, likely as a compensatory response to the energy deficit. These effects require the GAP-related domain, suggesting that Ras signaling is involved. Importantly, Nf1 regulates metabolism via effects in neurons, suggesting that neuronal control of metabolism is altered.

Loss of Nf1 in neurons drives behavioral alterations via effects during a developmental critical period. For instance, knockdown of Nf1 during development, but not adulthood, drives increased grooming, a major behavioral phenotype in flies. This developmental requirement for Nf1 raises the question of whether Nf1 affects metabolism via developmental mechanisms as well. To test this, we knocked Nf1 conditionally and examined the effect on metabolism. Using a neuron-specific driver (elav-Gal4) paired with a temperature-sensitive repressor (tub-Gal80^{ts}), we knocked Nf1 down during different developmental stages. Metabolism was examined in flies using respirometry to measure CO_2 production. Nf1 was either knocked down throughout life or restricted to one several developmental stages: early development (embryo through second instar larvae), late development (third instar larvae through pupal stage), or adult (following eclosion). Knocking down Nf1 selective during late development increased CO_2 production in adults. These data suggest that normal metabolic function in adults requires Nf1 during a developmental critical period.

500F **Modeling a MOPD II patient mutation in Pericentrin reveals tissue-specific centrosome effects** Makenzie S Thomas, Brian J Galletta, Jacob Ortega, Nasser Rusan National Heart, Lung, and Blood Institute, National Institutes of Health

Centrosome dysfunction is heavily linked to developmental conditions that affect brain and body size, including primordial dwarfism and microcephaly. However, the cellular mechanisms implicated in these conditions remained poorly defined. In this study, we investigate a mutation in *Pericentrin,* a centrosome-associated protein, identified in a subset of families with Microcephalic Osteodysplastic Primordial Dwarfism type II (MOPD II). Unlike typical *Pericentrin* mutations that cause severe protein truncation, this mutation involves a single lysine deletion, offering a unique opportunity to explore Pericentrin function in the context of MOPD II. Using *Drosophila* as a model, we examined the tissue-specific effects of *Pericentrin-like protein* (PLP) carrying an equivalent deletion, ΔR2720 (hereby $plp^{\Delta R}$). As it is known that PLP is critical for the recruitment of pericentriolar material (PCM), proper formation of motile cilia during spermatogenesis, and the construction of sensory organ cilia, we hypothesize that $plp^{\Delta R}$ may impair one or more of these processes by disrupting a subset of known protein interactions. Our results demonstrate that $plp^{\Delta R}$ reduces PCM levels ~25% in symmetric and asymmetric divisions, leading to defects in centrosome organization. We are currently testing if this PCM reduction might alter mitosis and cell number. To explore whether $plp^{\Delta R}$ impacts sensory ciliogenesis, we performed behavioral assays that revealed defects in gravitaxis and mechanosensation, suggesting impaired sensory cilia function. Despite the effects of $plp^{\Delta R}$ on PCM and sensory behavior, spermatogenesis appears unaffected. These and other results suggest $plp^{\Delta R}$ is a hypomorphic allele that has tissue-specific effects related to the clinical manifestations of MOPD II such as reduced brain and body size and sensory deficits. Current experiments are focused on understanding the impact of $plp^{\Delta R}$ on neuroblast counts and basal body dynamics in sensory cilia. Finally, our yeast two-hybrid experiments reveal that $plp^{\Delta R}$ disrupts the interaction between PLP and Asterless (Asl), another centrosome-associated protein, while maintaining other known PLP interactions. This surprising result led to our hypothesis that the etiology of MOPD II could be the specific loss of the PLP-Asl protein interaction. Overall, our work offers new insights into the failed cellular mechanisms underlying MOPD II, potentially linking this human disease to the loss of a single protein-protein interaction.

501F **Behavioral correlates of intergenerational trauma in *Drosophila melanogaster*** Alyssa Davis, James Lee, Elizabeth Miller, Lucy Ramos, Shadman Murphy, Kevin Donaldson, Sarah G Clark Georgia State University

Intergenerational trauma is a poorly-understood phenomenon in which traumatic events experienced by one generation affect subsequent generations. Intergenerational trauma harms many groups of people extensively, thus it is vital to deepen our knowledge and understanding of the processes underlying it. *Drosophila melanogaster* is a model organism that procreates rapidly and can be exposed to a wide variety of experimentally validated high-stress situations. These qualities make *Drosophila* a promising organism to use for studying the effects of trauma passed between generations. Previous research has demonstrated epigenetic changes associated with intergenerational stress in *Drosophila*, therefore this research aims to identify and define behavioral changes due to intergenerational stress *Drosophila*. We present here the intergenerational impacts of trauma due to sleep deprivation, restraint stress, or social isolation on *Drosophila*, as measured by forced swim, open-field, and social space behavioral assays on first generation offspring.

502F **Functional conservation of the human *ATP1A3* in a *Drosophila* model of Alternating Hemiplegia of Childhood** Jennifer A O Ogbeta[1], Amanda Bretman[2], Elwyn Isaac[2], Steve J Clapcote[1] [1]School of Biomedical Sciences, University of Leeds, [2]School of Biology, University of Leeds

Alternating Hemiplegia of Childhood (AHC) is a rare and complex neurodevelopmental disorder characterised by infantile onset (<18 months) of episodic temporal paralysis affecting one or both sides of the body. These attacks are triggered by a range of external stressors and alleviated by sleep. AHC patients also experience movement, cognitive, behavioural and psychiatric symptoms that become more evident with age. The primary cause of AHC is heterozygous *de novo* mutation in the *ATP1A3* gene, encoding the neuron-specific α3 subunit of Na^+/K^+ ATPase (NKA). The NKA α3 subunit is responsible for the rapid restoration of basal intracellular Na^+ in neurons following sustained discharge. The study of AHC is heavily reliant on mouse usage. However, mechanical stress-induced paralysis displayed in a fruit fly line, carrying the fly equivalent (G774S) of the human G755S AHC mutation within its NKA α-subunit (*ATPalpha*), highlights the potential usage of a simpler and less expensive animal model for *in vivo* studies. Further characterisation of heterozygous G477S NKA α mutant flies revealed additional AHC-relevant phenotypes including heightened sensitivity to cold-induced paralysis, hypoactivity, and aberrant sleep behaviour, suggesting functional conservation between the ubiquitously expressed fly NKA α-subunit and the neuron-specific human NKA α3-subunit. Consistent with this, expression of the human wildtype *ATP1A3* rescues cold-sensitivity in G744S and null NKA α mutant flies. These data validate the use of *D. melanogaster* as a model system in the study of AHC.

503F **Tau did you forget? An investigation of native Drosophila tau in an Alzheimer's model** Carlie Epstein[1], Sarah Clark[2] [1]Georgia State University, [2]Neuroscience, Georgia State University

Many studies have used *Drosophila* to model Alzheimer's disease (AD) and investigate the behavior of both tau and beta-amyloid proteins. Studies investigating tau invariably use flies in which human tau is expressed, however, *Drosophila* has its own tau gene that generates a protein with 46% identity to human tau with conservation of the microtubule binding domains and of the Ser202/Thr205 phosphorylation sites that are used to identify hyperphosphorylated tau in AD (Heidary 2001). Thus, when researchers express human beta-amyloid and human tau in a fly and draw conclusions about the toxicity to neurons, changes in behavior, and impairments in memory function that result, they may be neglecting an additional factor: native *Drosophila* tau. We propose that it is possible that human beta-amyloid may induce changes in the behavior and phosphorylation state of *Drosophila* tau just as it does to human tau, and that if these changes are present, they should be considered in the interpretation of results from *Drosophila* models of AD.

Unfortunately, there are very few reagents available to investigate native Drosophila tau, so we are not able to directly assess changes in phosphorylation in the presence of beta-amyloid. We present here the results of a study in which adult female *Drosophila* with GFP-tagged *Drosophila* tau are crossed to adult male *Drosophila* expressing human beta-amyloid (wild-type or familial AD "Arctic" mutation) via the pan-neuronal nSyb-Gal4 driver. The larvae produced from these crosses are dissected and imaged to document tau localization patterns in sensory neurons, determining whether interactions with beta-amyloid might be changing native *Drosophila* tau's microtubule-binding behavior. We also compare via Western blot the amount of native tau present in the brain at three developmental stages (third instar larva, young adult, and aged adult) in wild-type flies, flies expressing unmutated human beta-amyloid, and flies expressing human beta-amyloid with the Arctic mutation.

504F **Flies with alleles of TDP-43 that cause familial ALS experience sustained DNA damage after X-ray irradiation.** Samantha N Cobos[1], Joshua Dubnau[1,2] [1]Neurobiology and Behavior, Stony Brook University, [2]Anesthesiology, Stony Brook University

Amyotrophic lateral sclerosis and frontotemporal dementia (ALS/FTD) are devastating neurodegenerative conditions. One of the key pathophysiological hallmarks of ALS and FTD is loss of nuclear localization and abnormal cytoplasmic aggregation of TAR DNA-binding protein 43 (TDP-43). Such pathology is seen in the vast majority of ALS and a substantial fraction of FTD cases. Although most cases of ALS/FTD are sporadic, with no known genetic cause, a small fraction of ALS/FTD cases are caused by mutations in the *TARDBP* gene, which encodes the TDP-43 protein. TDP-43 is a highly pleiotropic RNA and DNA binding protein that shuttles between the nucleus and cytoplasm, although most of the TDP-43 protein is normally nuclear. TDP-43 plays roles in many aspects of RNA metabolism and gene expression, including splicing, mRNA translation, and silencing of retrotransposons (RTEs) and endogenous retroviruses (ERVs). The regulation of RTEs/ERVS has been of particular interest to our group because we have shown that both the fly mdg4-ERV and human HERV-K are not only de-repressed in response to TDP-43 aggregation, but also can trigger TDP-43 protein aggregation when they are expressed. Thus TDP-43 aggregation and ERV expression exist in a positive feedback loop. Expression of ERVs and aggregation of TDP-43 also are each associated with accumulation of DNA damage. TDP-43 protein is normally recruited to sites of DNA damage and is involved in repair of double stranded breaks via non-homologous end joining (NHEJ), which is the favored DNA DSB repair mechanism available in post-mitotic neurons. We therefore decided to investigate the effects of introducing DNA damage to adult flies that express either wild type human TDP-43 or familial TDP-43 alleles that are causal of ALS human subjects.

Here, we compared the effects of X-ray induced DNA damage in "humanized" *Drosophila* in which the human TDP-43 is knock-in to replace the fly ortholog. We find that familial disease-causing alleles of human TDP-43 displayed abnormal repair upon X-ray-induced DNA damage. We find, for example, that hTDP-43$^{M337V\text{-}KI}$ flies exhibit sustained DNA damage throughout the brain even 10 days post-irradiation. By contrast, control flies that express only the fly ortholog, or those that express the hTDP-43$^{WT\text{-}KI}$, exhibit only transient damage after irradiation. This provides a platform to elucidate the mechanisms by which TDP-43 mutations lead to dysfunctional DNA damage repair.

505F **Investigating variants associated with *HNRNPH2*-related neurodevelopmental disorder using *Drosophila* models** Melanie Mew[1,2], Jonathon Andrew[1,2], Bandana Sharma[1,2], Blake Vuocolo[1,2], Sharayu Jangam[2,3], The Texome Project[1], Michael Wangler[1,2,4] [1]Molecular and Human Genetics, Baylor College of Medicine, [2]Jan and Dan Duncan Neurological Research Institute, [3]Baylor College of Medicine, [4]Department of Pediatrics, Baylor College of Medicine

Glorund (Glo) is an essential RNA binding protein with known roles in RNA processing, and *glo* corresponds to five human orthologs in the Heterogeneous Nuclear Ribonucleoprotein (HNRNP) family. One of these orthologs—*HNRNPH2*—is associated with a rare neurodevelopmental disorder termed HNRNPH2-NDD. Previous research using cell and mouse models found that *HNRNPH2* variants can cause protein localization defects and altered transcriptomic profiles. However, these models fail to reveal the mechanism driving HNRNPH2-NDD pathogenesis due to functional interference from HNRNPH1, a mammalian paralog that is 96% sequence identical to HNRNPH2. Because *glo* is the single fly ortholog for both *HNRNPH1* and *HNRNPH2*, we are able to explore the effects of *HNRNPH2* variants in a paralog-free background. We have generated fly models which overexpress *HNRNPH2-reference* (*ref*) or *variant* (*var*), and we are also characterizing *glo* knockdown (KD) and overexpression (OE) phenotypes. Here, we present evidence that *glo* expression is necessary for development and survival in a dosage-sensitive manner, as KD or OE of *glo* using ubiquitous drivers is associated with lethality. We observe *glo* expression in a subset of neurons in the larval and adult CNS, and we found that *glo* KD using an early neuron-specific driver is lethal, while KD with a late neuron-specific driver does not affect viability but does impair climbing ability. This suggests that *glo* expression in developing neurons is essential for survival and that expression in mature neurons is functionally important. In addition, our data show that ubiquitous, pan-neuronal, or pan-glial OE of *HNRNPH2* constructs is lethal, while OE with eye-, wing-, and thorax-specific drivers shows more severe phenotypes for *HNRNPH2-var* compared to *-ref*. These findings strongly support that the variants modeled here—one of which is a previously unreported variant-of-unknown-significance—are pathogenic. Interestingly, eye-specific phenotypes are consistent with known patterns of altered JAK/STAT signaling in *Drosophila*. We are actively working to investigate conserved pathways through which *HNRNPH2* variants cause disease, including possible effects on members of the JAK/STAT pathway. These findings underscore the utility of using *Drosophila* models to investigate the phenotypic consequences of variants associated with human disease, and our work to dissect patterns of aberrant cell signaling may uncover novel therapeutic options for HNRNPH2-NDD.

506S **Assessing the Role of Kefir in Climbing Ability in a Parkinson's Disease Model** Sierra M. Hartland[1], Ari Tendo[1], Rachael B. D. Triglia[1], Kathryn A Jewett[2] [1]Juniata College, [2]Biology, Juniata College

Parkinson's disease (PD) is a neurodegenerative disorder characterized by the loss of motor skills, memory deficits, and irregular protein aggregation. Recent research has shown that the gut microbiome and dietary choices can significantly impact overall health and could potentially play a role in managing PD symptoms. Probiotics, such as kefir, have the potential to improve mobility and sleep patterns in patients with PD. We are using a *Drosophila melanogaster* model to investigate how dietary manipulation with kefir influences Parkinson's-like symptoms. We will assess the effect of kefir treatment on climbing ability in both healthy flies and sick flies carrying a deletion of the *GBA* gene which is associated with PD. Flies were collected immediately after eclosion and divided into three experimental food groups: water (control), kefir, and diluted kefir. Flies were flipped onto new food every other day for 10 days before the climbing assay. After the climbing assay, flies were frozen for western blotting of protein aggregation.

507S **Characterizing effects of expressing human CDK19 patient variants in a *Drosophila melanogaster* Cdk8 depleted background** Karampal S Grewal, Jenny Zhe Liao, Esther M. Verheyen Molecular Biology and Biochemistry, Simon Fraser University

Cyclin dependent kinases regulate cell cycling, gene expression, and more recently illustrated, mitochondrial dynamics. Depletion of Drosophila cyclin dependent kinase 8 (*dCdk8*), the fly ortholog to human *CDK8* and *CDK19* (*hCDK19*), presents with adult wing phenotypes, thicker muscle myofibrils, fused mitochondria in muscle tissue and climbing defects. The expression of wild type *hCDK19* in *dCdk8* knockdown flies rescues these defects. In recent years, numerous heterozygous missense mutations have been characterized in *hCDK19*. Patients harboring these variants present with global developmental delay, intellectual disability and other muscular and neurological deficiencies, indicative of a dominant effect of these variants. Two such variants described in Chung et *al.* (2020), $CDK19^{Y32H}$ and $CDK19^{T196A}$, and other *CDK19* variants discovered since then, are linked to a syndrome classified as Developmental Epilepsy and Encephalopathy 87 (DEE-87). Upon initial discovery, these two variants were hypothesized to act in a dominant negative fashion. The broad clinical assessment at that time was accompanied with a subset of specific model organism experiments using Drosophila. In these experiments the fly ortholog *dCdk8* was knocked down while expressing the patient *hCDK19* variants using the Gal4/UAS system. Following the work of Chung et al., Zarate and co-workers expanded our understanding by showing that Y32H is likely a gain of function through elevated kinase activity and proposed that T196A is a loss of function through reduced kinase activity, though untested. We present data from our ubiquitous expression model to test the activity and functionality of these variants in a *dCdk8* depleted background, including examining sex differences in our analyses. We assessed fly lifespan, locomotion, and muscle fiber and mitochondrial morphology in both males and females. We show that in a ubiquitous *dCdk8-RNAi* knockdown, ubiquitous expression of Y32H can produce a rescue of the *dCdk8* knockdown phenotype through a possible gain of function compensatory effect, while T196A is unable to do so through a possible loss of function in kinase activity. Our studies in flies allow us to assay these variants in numerous contexts to gain further insight into their function and obtain translational knowledge to apply back to human health.

508S **Investigating the combined effects of epithelial tumors and hypoxia on whole-body physiology** Shahoon Khan, Anissa Mourali, Michael Turingan, Abhishek Sharma, Savraj Grewal University of Calgary

As tumors develop, they not only disrupt the function of the organs they grow in but also frequently exert effects on distal tissues. These cell non-autonomous effects of tumors often manifest as complex pathological conditions including disrupted immune signaling, renal dysfunction, and nutrient and tissue wasting. Understanding host-tumour interactions is, therefore, a crucial aspect of cancer research. One key determinant of a tumor's biology is its local microenvironment, often characterized by low oxygen levels, known as hypoxia. Although hypoxia is deleterious for normal cells, tumour cells carry genetic mutations that allow them to thrive in hypoxic environments. As a result, hypoxic tumour microenvironments are often associated with aggressive cancers, metastasis, and poor prognosis. While cell culture studies have defined how hypoxia cell autonomously influences tumour progression, it is unclear how hypoxia shapes the cell non-autonomous effects of tumours on host physiology. Our work utilises *Drosophila* larvae to address this gap. Our lab discovered that when we generated epithelial tumors by genetically over activating the oncogenic Target of Rapamycin (TOR) pathway in *Drosophila* larvae, it led to tissue overgrowth. Interestingly, when these animals were raised in hypoxic conditions, tumor-bearing larvae displayed reduced body growth, delayed maturation to the pupal stage, and suppressed survival. We show that these growth suppressing effects occur after a key developmental checkpoint known as critical weight (CW), which assesses larval nutrient stores to ensure the animals' survival during the non-feeding pupal phase. After CW, the combination of epithelial tumors and hypoxia were found to exert systemic, cell non-autonomous effects to deregulate nutrient stores and suppress distal tissue growth. Analyzing the gene expression of hypoxic tumors showed upregulation of various tumour-derived cytokines, including Drosophila insulin-like peptide 8 (Dilp8), a factor previously shown to delay larval maturation and suppress distal tissue growth, suggesting a mechanism that hypoxic tumours can use to exert systemic effects. Given that the cellular signalling pathways that govern hypoxic responses, tumorigenesis, and systemic growth are highly conserved, our work provides insights into how hypoxia influences the cell non-autonomous effects of tumours.

509S **Effect of Muscle Cell-Specific Glucocerebrosidase Expression on Protein Aggregation and Sleep Regulation in *Drosophila melanogaster*** Regan L Farringer, Kathryn A Jewett Juniata College

Parkinson's disease is the second most prevalent neurodegenerative disorder, characterized by movement difficulties, tremors, and balance issues. It is often linked to mutations in the gene responsible for producing glucocerebrosidase (GBA). In *Drosophila melanogaster*, mutations in the *GBA* gene lead to symptoms resembling Parkinson's disease, such as changes in sleep patterns, increased protein aggregation, shortened lifespan, and reduced climbing ability. This project aims to investigate how muscle-specific expression of *GBA* influences sleep disturbances and overall protein aggregation. We will compare four groups of flies: those lacking the *GBA* gene (deletion), those with normal *GBA* expression (control), those missing the *GBA* gene but with ectopic expression in muscle cells using the *GAL4/UAS* system (rescue), and those with both normal *GBA* expression and ectopic muscle cell expression (overexpresser). Flies will be aged and then frozen on day 10, a point at which Parkinson's-like symptoms are significantly pronounced in the deletion flies. Protein aggregation will be assessed using western blot. We anticipate that the deletion flies will show higher levels of protein aggregation compared to the rescue flies, which should resemble the control group. Additionally, we will monitor their sleep patterns using the Drosophila Activity Monitoring System. We expect to find that the rescue flies exhibit fewer sleep disturbances than the deletion group, indicating a neurological effect driven by *GBA* expression in muscle cells, as previous research has shown non-cell autonomous effects in this model.

510S **The Role of Glial Glucocerebrosidase in Parkinson's Disease Pathogenesis: A Study in *Drosophila*** Stephen kataria, Kathryn A Jewett Juniata College

Mutations in the glucocerebrosidase (GBA) gene are a prominent risk factor for Parkinson's disease (PD). While GBA's neuronal role in PD pathogenesis is established, its function in glial cells remains poorly understood. To investigate the glial-specific contribution of GBA to PD, we utilized a *Drosophila melanogaster* model. GBA-deficient (*Gba1b*) flies exhibit hallmark PD phenotypes, including reduced lifespan, locomotor deficits, and accelerated protein aggregation, indicative of neurodegeneration. These mutants also display disrupted sleep architecture, characterized by reduced total sleep, particularly during the night, and impaired sleep recovery after deprivation. Given the emerging role of glia in neuronal function and the non-cell-autonomous effects of GBA, we hypothesize that glial GBA expression modulates neuronal health and sleep regulation. To test this, we employed the *UAS/GAL4* system to generate flies with targeted GBA expression in glial cells. Four experimental groups were established: 1) wild-type controls; 2) *Gba1b* mutants; 3) *Gba1b* mutants with glial-specific GBA rescue; and 4) glial-GBA overexpressing controls. This approach allows for the dissection of GBA›s glial-specific effects. We will analyze sleep architecture and protein aggregation in the heads and bodies of these flies. Future experiments will assess locomotor function through climbing assays. We anticipate that our findings will elucidate the role of glial GBA in neuroprotection and sleep regulation. This study may provide mechanistic insights into how GBA mutations contribute to PD pathogenesis, particularly with respect to sleep disturbances, a common non-motor symptom. Ultimately, understanding GBA›s diverse functions in glial cells may reveal novel therapeutic targets for GBA-associated neurodegenerative disorders.

511S **Understanding how Zika Virus Targets Glucocerebrosidase (GBA) During Neurodevelopment** Uchechukwu E Mgbike[1], Adriana Bibo[1], Adam Fishburn[2], Priya Shah[3], Nichole Link[1] [1]Neurobiology, University of Utah, [2]Microbiology and Molecular Genetics, University of California, Davis, [3]Chemical Engineering & Microbiology and Molecular Genetics, University of California, Davis

Zika virus (ZIKV) is a single-stranded RNA virus belonging to the Flaviviridae family along with Dengue and West Nile virus. Infections in adults can be mild and patients often recover. However, in the developing foetus, this infection can be severe leading to a range of phenotypes clinically termed Congenital Zika Syndrome (CZS). CZS causes severe neurodevelopmental disorders such as microcephaly, seizures, muscle pains and cognitive defects, none of which are curable. To understand how ZIKV affects development and causes microcephaly, it is essential to determine the molecular pathways that ZIKV hijacks during infection. We previously showed that *Drosophila* is an excellent model to investigate how ZIKV causes disease. Expression of ZIKV non-structural protein 4B (NS4B) in the developing brain causes a microcephaly phenotype. Protein-protein interaction (PPI) data show that NS4B interacts with human glucocerebrosidase (hGBA). GBA is a lysosomal enzyme that regulates the degradation of glycosphingolipids and glucosylceramides needed for normal brain function. Mutations in this gene causes both neuronopathic and non-neuronopathic forms of Gaucher's disease. We use *Drosophila melanogaster* to validate the NS4B-GBA interaction *in vivo* using tissue-specific UAS-GAL4 expression system. While NS4B expression alone induces lethality and small brains, we show that co-expression of hGBA with NS4B rescues both phenotypes. We now aim to deduce the functional significance of the NS4B-GBA interaction during neurodevelopment and infection. Since there are no effective vaccines or treatments for the viral infection and neuronopathic forms of Gaucher's disease, findings from this study can contribute to therapy and drug development to combat the virus.

512S **An interorgan wound response is hijacked by tumors to promote chronic, lethal intestinal inflammation** Katy Ong, Karina Anders, David Bilder UC Berkeley

Dysregulated endocrine signaling between tumors and distant organs contributes to cancer morbidity and mortality through poorly understood signaling axes. In a *Drosophila* ovarian carcinoma model, tumor-bearing flies die 50% earlier, but the cause of death has been elusive and occurs in the absence of metastasis. We previously found that this model displays profound blood clotting defects (coagulopathy), and attenuation of coagulopathy improved the lifespan of tumor bearing flies. In searching for the physiological basis of this rescue, I now show that tumor-bearing flies experience significant gut inflammation. This inflammatory response is characterized by intestinal stem cell dysregulation, epithelial dysplasia, persistent activation of stress pathways (Jak/STAT and JNK), and failure of the gut barrier, all of which are decreased when coagulopathy is experimentally reduced. These findings suggest that tumors initiate a systemic wound response in the intestinal epithelium, and clotting dysregulation by the tumor exacerbates this response. I will discuss the signaling events involved in this pathological interorgan signaling axis, which converts an adaptive homeostatic reaction into chronic, maladaptive inflammation, as well as how tumor-host biology provides insights into conserved pathways in systemic inflammation and wound healing.

513S **A *Drosophila* tumor model to unravel how older animals are uniquely challenged by cancer** Jan Mikhale B Cajulao, David Bilder Molecular and Cell Biology, University of California, Berkeley

Age is a significant risk factor for most diseases, including cancer. While the accumulation of somatic mutations throughout life increases the likelihood of tumorigenesis, interactions with the tumor microenvironment and distant non-transformed tissues also play critical roles in tumor progression and host mortality. How the latter phenomena change during aging is unclear, in part because most tumor models utilize young animals. *Drosophila* are short-lived but share many hallmarks of aging as well as cancer with mammals, including clinically relevant effects on host tissues such as cachexia, clotting defects, and immune cell recruitment. This suggests that the short-lived fly may be a productive system to investigate how the host response to tumors changes with age. By utilizing an inducible system to drive oncogenes in the ovarian epithelium, I can compare flies with tumors induced in young versus old flies. Surprisingly, older tumor-bearing flies exhibit delayed mortality compared to their younger counterparts, depending on the genetic background. Tumors grown in old flies also progress more slowly. Remarkably, there are higher numbers of associated hemocytes in old tumors despite a reduction in total hemocyte numbers throughout the fly's lifetime, suggesting the anti-tumor immune response changes with age. I will present my continuing work exploring why old tumors progress slower.

514S **Neuronal expression of the Amyloid α peptide ($A\beta_{17-42}$) in *Drosophila* has deleterious effects on lifespan, behavior, degeneration, and gene expression and exacerbates the effects of full-length $A\beta_{1-42}$** Hannah Moalla[1], Rebekah I Larreynaga[2], Ginger Holmer[3], Roktima Godhuli[3], Guillermo Martinez[3], Emily Broutian[3], Jazmin Chavez[3] [1]University of California, Santa Cruz, [2]Molecular, Cell, and Developmental Biology, UC Santa Cruz, [3]UC Santa Cruz

Alzheimer's disease (AD) is a neurological disorder caused primarily by the accumulation of neurotoxic aggregates, leading to gradual cognitive dysfunction and, in some cases, loss of mobility skills. About 1 in 9 people in the U.S. over the age of 65 are affected by AD.

Amyloid plaques form when a transmembrane amyloid precursor protein (APP) is cleaved by secretases, forming Amyloid-β ($A\beta_{1-42}$). This peptide aggregates, eventually forming plaques between neurons. This leads to neurodegeneration and cognitive decline.

Amyloid-β_{17-42} ($A\beta_{17-42}$), also known as Amyloid-α (Aα) or p3, is an alternative product of APP cleavage. Aα has recently been shown, like Aβ, to be amyloidogenic in vitro. Current research on AD has emphasized the role of Aβ in neurodegeneration. We aim to examine Aα's potential involvement in AD. Experiments in transgenic *Drosophila* have shown Aα has degenerative effects similar to, but less severe than, Aβ and that co-expression of Aα with Aβ exacerbates Aβ's degenerative effects.

To assess the neurodegenerative effects of Aα and Aβ, a climbing assay and a longevity assay were conducted on flies expressing Aα and Aβ separately and together. Scanning electron microscopy (SEM) was used to visualize effects on the developing eye. The results of these assays indicated that Aβ and Aα have deleterious effects on locomotive ability, overall lifespan, and ommatidia and bristle structures, with the most significant degenerative effects seen in flies co-expressing Aβ and Aα. These data suggest that Aα exacerbates the effects of Aβ and has similar but less severe degenerative effects on its own. RNA sequencing shows that Aβ- and Aα-expressing flies both downregulate genes involving proteolysis, while Aα/Aβ co-expressing flies upregulate these genes. Staining of imaginal eye discs to assess the expression of the pro-apoptotic genes DCP-1 and MMP1 showed that both Aβ- and Aα-expressing flies have higher levels of apoptosis in developing neurons than non-expressing flies. These experiments further show Aα's similar but less severe degenerative effects compared to Aβ.

We will perform co-immunoprecipitation and immunohistochemistry assays to assess whether Aα directly interacts with Aβ in *vivo* and whether Aα contributes to Aβ aggregation, potentially explaining the increased deleterious effects in our co-expression experiments. These studies aim to enhance our understanding of the molecular interaction between the peptides and Aα›s role in AD pathology.

515S **Tethered Fly Electrophysiology Reveals Alterations in Seizure Expression Associated With Dietary ω-3 Fatty Acids in *para*^{Shu}, a *Drosophila* Na$_v$ Channel Gain-of-Function Mutant** Reid Schuback[1], Victoria Hand[1], Saul Landaverde[1], Toshihiro Kitamoto[2], Atulya Iyengar[1] [1]Department of Biological Sciences, The University of Alabama, [2]Department of Neuroscience and Pharmacology, The University of Iowa

Mutations in voltage-gated sodium (Na$_v$) channel genes are linked with several epilepsy syndromes and related neuronal excitability disorders. Seizures in patients carrying such mutations are often uncontrolled and pharmacoresistant. In flies, Na$_v$ channels are encoded by a single gene, *paralytic* (*para*). A gain-of-function allele, *para*^{Shu}, displays spontaneous spike bursts characteristic of seizures, along with convulsions exacerbated by high temperature. Remarkably, several behavioral phenotypes in *para*^{Shu} flies are suppressed by dry milk, milk whey or an ω-3 fatty acid component, alpha-linolenic acid (ALA) in the diet (Kasuya et al. 2019; 2023). Both dry milk and milk whey furthermore attenuate electrophysiological seizure-related phenotypes in *para*^{Shu}. However, it is unclear whether ALA supplementation alone is sufficient for the effect. Here, we compare the effects of milk whey (2.0% w/vol) and ALA (0.05 % w/vol) on the expression of spontaneous and electrically evoked seizures in *para*^{Shu} mutants. Using a tethered fly preparation and tungsten electrodes, we monitored spiking in dorsal longitudinal muscles (DLMs). Seizures manifest as abnormal high frequency firing synchronized bilaterally. We found *para*^{Shu}/Y flies displayed intermittent periods of rhythmic DLM firing (~8 Hz) punctuated with high-frequency bursts (~ 20 Hz), while wild-type (CS) flies only displayed sparse, grooming-related, spikes. In both milk whey and ALA-fed flies, the rhythmic firing component was often observed, while the bursts were largely absent. Heterozygous *para*^{Shu}/+ females mostly displayed grooming-related spiking patterns, although 10/24 individuals also showed occasional lower frequency (~4 Hz) abnormal flight-like firing events uncoupled from wing movements. The proportion of whey and ALA-fed flies displaying this firing pattern decreased substantially (1/9 and 4/19 respectively). Lastly, we examined electroconvulsive seizure (ECS) activity in *para*^{Shu}/+ and wild-type flies fed the respective diets. Both whey and ALA supplementation increased the threshold to induce seizure activity and made characteristic modifications to the ECS firing pattern in *para*^{Shu}/+ mutants. Interestingly, wild-type counterparts also displayed alterations in the stereotyped ECS sequence following whey and/or ALA supplementation. Together, our results indicate dietary supplementation of milk whey and ALA engage similar mechanisms to suppress seizure phenotypes in *para*^{Shu} mutants.

516S **Characterizing Alzheimer's in a *Drosophila* Model Carrying Synthetic Mutations of Amyloid Beta (Aβ)** Jordan Sitea[1], Olivia Nardell[1], Kaliah Wood[1], Brett Chan[1], Aahwan Koirala[1], Jorge Moran[1], Caroline Yu[1], Jesus Quiroz[1], Jennifer Ly[1], Bailey Thompson[1], Arushi Garg[1], Kaia Levy-Kanenaga[1], Maria Sajimon[2], Jevgenij Raskatov[2], Jeremy Lee[1] [1]Molecular, Cell, and Developmental Biology, University of California, Santa Cruz, [2]Chemistry and Biochemistry, University of California, Santa Cruz

Alzheimer's Disease (AD) is a progressive neurodegenerative disorder affecting over 55 million people globally. A hallmark of AD is the accumulation of amyloid beta (Aβ) plaques. AD is characterized by disruption of neuronal communication and eventual neurodegeneration, which drives disease progression. Research in our lab using *Drosophila* models expressing wild-type (WT) Aβ pan-neuronally has demonstrated shortened life spans, behavioral deficits, reduced locomotor capabilities, and changes in gene expression, mirroring aspects of AD pathology. Dominant inheritance of specific Aβ amino acid substitutions in early-onset AD suggests that single-point mutations can affect Aβ aggregation and neurotoxicity.

Work in Dr. Jevgenij Raskatov's lab has identified two $A\beta_{42}$ side chain modifications (Q15E, N27D, and a double mutant 2Δ) that each reduces its aggregation kinetics and cytotoxicity *in vitro*. We have incorporated these modifications in $A\beta_{42}$ as missense mutations in transgenic *Drosophila*, allowing for *in vivo* characterization of the effects of these single amino acid changes on $A\beta_{42}$'s deleterious effects. Using the GAL4-UAS system, we are conducting assays to compare the phenotypes of flies expressing WT $A\beta_{42}$ and flies expressing these mutant forms of $A\beta_{42}$.

To visualize morphological differences in neuronal development, we use Scanning Electron Microscopy (SEM) to examine eye morphology in mutant flies and quantify the results by comparing them to flies expressing WT $A\beta_{42}$ and non-expressing controls. Preliminary results indicate that 2Δ expressing flies have fewer morphological abnormalities than Q15E or N27D, which had fewer than WT $A\beta_{42}$ expressing flies. PCR has confirmed the presence of our mutant inserts. RT-PCR will confirm mutation expression. A longevity assay will assess the survivorship of flies expressing the mutant $A\beta_{42}$ panneuronally. Locomotor decline will be measured with a Rapid Iterative Negative Geotaxis (RING) assay to compare mutants with WT and control groups.

This research aims to advance the understanding of AD by identifying how specific $A\beta_{42}$ structural modifications affect aggregation and toxicity, potentially increasing our understanding of the mechanism(s) by which $A\beta_{42}$ negatively affects neuron function and inform future therapeutic strategies.

517S **Discovery of small molecule modulators for PLP1-related disorders via a drug repurposing screen** Delaney Baratka, Katherine Beebe, Emily Coelho, Caroline Massey, Heather D. Evans, Clement Y. Chow University of Utah

Proteolipid proteins are a conserved family of proteins that function in cell membranes. In humans, mutations in the gene encoding the myelin proteolipid protein PLP1 lead to a range of X-linked neurodegenerative disorders known as PLP1-related disorders. One such disorder, spastic paraplegia 2 (SPG2), results from loss-of-function mutations in *PLP1* and is characterized by progressive symptoms, including lower limb hypertonia and ataxia. The *Drosophila* ortholog of *PLP1* is *M6*, the sole proteolipid protein in *Drosophila*. Previous studies established a role for *M6* in the eye and ovary. Our research goal was to use an *M6* loss-of-function eye phenotype and perform a small molecule screen to identify candidate compounds for proteolipid protein-based disease in humans. Using a drug repurposing approach, we screened a library of 1520 FDA- and EMA-approved compounds to identify those capable of rescuing the $M6^{RNAi}$ small-eye phenotype. The primary screen identified 33 suppressor compounds, which improved the eye phenotype, and 39 enhancer compounds, which worsened it. Follow-up experiments focused on 5 drugs of clinical interest, some of which share structural similarities. Current studies are aimed at understanding the mechanism by which these compounds rescue $M6^{RNAi}$ phenotypes in the eye. Additionally, we are extending our studies to assess the effects of *M6* loss and drug rescue in neuronal and glial cells.

518S **Differential immune responses may contribute to varying outcomes between a single, severe TBI and a mild, repeated TBI** Daniel Tulchinskiy[1], Jorge A Garcia[1], Kamden Kuklinski[1], Doyinsola Ogunshola[1], Otoha Tatami[1], Maria Jose Orozco Fuentes[1], Rebecca Delventhal[2] [1]Lake Forest College, [2]Biology, Lake Forest College

Traumatic brain injuries (TBIs) occur when external forces damage the brain, commonly resulting in hospitalization, long-term disability, and death. This neurological disorder accounts for nearly 30% of deaths due to injury in the US. It is challenging to comparatively study the consequences of different patterns of head injuries in the human population due to varying injury characteristics, and patient demographics. To mitigate these confounding variables, we used *Drosophila melanogaster* as a model organism to study the short- and long-term outcomes of mild, repeated TBI (multi-day, MD) compared to a single, severe TBI (single-day, SD). We discovered that the outcomes of the two patterns of TBI differed such that flies in the MD condition performed better or similar to flies in the SD condition short-term but showed worse outcomes long-term. We found that flies given a MD TBI showed lower acute mortality (within 48 hours), but the surviving flies displayed a shorter lifespan than flies given a SD TBI. Likewise, flies given a MD TBI exhibited worse long-term climbing ability. We hypothesized that different immune responses to MD versus SD TBI may mediate differences in short- and long-term outcomes and found evidence of prolonged immune gene expression MD TBI weeks after TBI. To determine if the differences in immune response were causal, we examined loss of function mutants for key immune genes and measured their outcomes following the two injury paradigms. We found that short- and long-term survival in both injury conditions worsened with a loss of Imd signaling, suggesting that Imd is likely protective for both MD and SD TBI. Interestingly, mutants lacking Toll immune signaling exhibited improved outcomes, only in the MD condition: lower acute mortality and longer lifespan relative to wild type controls, suggesting that Toll signaling may be detrimental for both short- and long-term outcomes from a MD TBI. Understanding whether there are differences in cell-specific immune responses and timing of these responses to different types of TBI are important next steps. This research could eventually inform the development of treatments tailored to specific injury patterns, ultimately improving outcomes.

519S **A Drosophila study identifies iPLA2-VIA as potential novel chemoprevention target for HPV-induced cancer** Sagarika Das, Rami N. Hassan, Mojgan Padash Barmchi Biological Sciences, University of Oklahoma

High-risk human papillomaviruses (HR-HPVs) are responsible for almost all cervical malignancies as well as considerable proportion of vaginal, vulvar, penile, and oropharyngeal cancers worldwide. Current treatments for cervical cancer are limited to radiotherapy and chemotherapy and besides an immunotherapy-based therapeutic for advanced stages of cancer, there is no molecularly-targeted therapeutic as standard-of care for cervical cancer treatment or prevention. Therefore, there is a critical need for development of novel molecularly-targeted interventions. HR-HPVs function by persistent expression and action of two viral oncogenes E6 and E7. E6 with the assistance of human E3 ubiquitin ligase (hUBE3A) targets several cellular proteins including tumor suppressor protein, p53 and select members of PDZ domain containing proteins for proteasomal-mediated degradation. For development of novel molecularly-targeted therapies, understanding the actions and mechanisms of E6 and E7 is crucial. Using a Drosophila model of HPVE6 plus hUBE3A and performing a large-scale deficiency screening we identified the calcium-independent phospholipase A2 VIA (iPLA2-VIA), as a gene whose single deleted copy suppresses morphological defects caused by co-expression of E6 and hUBE3A. These results were confirmed using two null alleles of iPLA2-VIA. We show that reduction of iPLA2-VIA suppresses E6+hUBE3A-induced perturbed ommatidial organization and proteasomal degradation of PDZ domain protein Magi. We further demonstrate that E6+hUBE3A expression alters the level of iPLA2-VIA in the mitochondria and leads to mitochondrial deficiencies and ROS release. Further analysis revealed that E6 alters the level and localization of both protein isoforms of iPLA2-VIA, PA and PB. We find that the PA isoform that is predominantly cytoplasmic translocate to mitochondria and the PB isoform which is predominantly mitochondrial is present as cytoplasmic aggregates and punctuated. These results suggest that iPLA2-VIA is likely to play a role in E6-induced mitochondrial deficiencies. To gain insight into the role of iPLA2-VIA in E6-associated cellular perturbation, we are currently conducting immunoprecipitation experiments to identify the interactors of PA and PB protein isoforms in E6+hUBE3A-expressing cells. Furthermore, given the role of iPLA2-VIA in lipid metabolism and membrane homeostasis we have performed lipidomic and are currently in the process of data analysis. Given that the human homolog of iPLA2-VIA, PLA2G6, can functionally replace its Drosophila counterpart, suggests a conserved mechanism of action. Hence, the Drosophila studies are combined with mammalian cell line studies for validation of Drosophila findings and to further demonstrate the potential of iPLA2-VIA as novel chemoprevention target for cervical cancer.

520S **Assessing Muscle Protein Ubiquitination during Tumor Induced Wasting in *Drosophila melanogaster*** Gabrielle Daughenbaugh[1], Morgan Marsh[2], Mardelle Atkins[2] [1]Biological Sciences, Sam Houston State University, [2]Sam Houston State University

Using *Drosophila Melanogaster,* we are studying the biological process of muscle wasting, also known as cachexia, in fruit fly larvae with induced tumors. Cachexic wasting is thought to be due to a metabolic imbalance with a shift to catabolism. A major driver of this catabolism is thought to be Ubiquitin mediated proteasomal degradation, as evidenced by elevated poly-ubiquitin observed by Western blotting from cachectic tissue. To validate if this is true in our model, we examined poly-ubiquitin using immunohistochemistry and Western blot. Intriguingly, we do not observe consistent presence of cytoplasmic aggregates of poly-ubiquitin, as expected. However, we do observe consistent perinuclear accumulation of poly-ubiquitin, which may allude to a more regulated process than wholesale proteasomal degradation. These results add to our understanding of the role of ubiquitination during tumor induced wasting.

521S **Investigating Changes in Activity and Circadian Rhythm in a Drosophila Model of Frontotemporal Dementia** Kendall Eby, Braeden Shields, Sarah Morley, Isabella DelNegro, Marla Tipping Providence College

Frontotemporal dementia (FTD) is a neurodegenerative disorder that affects behavior, personality, motor activity, speech, cognition, and sleeping patterns. This disease has many molecular etiologies, but the most common is the C9orf72 hexanucleotide expanded repeat. In FTD patients carrying this mutation, disruptions of circadian rhythm have been observed. We are investigating the cause of these disruptions by studying the expression of clock genes (Per and Tim). To this end, we are examining whether the disruptions observed in FTD patients also occur in a Drosophila model of the disease. Our research is focused on understanding if the disruptions are caused by irregular expression of the clock genes or by other factors. We conducted quantitative polymerase chain reaction (qPCR) analyses of clock gene expression in Drosophila to assess the impact of the disruptions at various time points to identify patterns corresponding to sleep-wake cycle changes. We compared this data with our behavioral analyses of Drosophila activity during a normal daylight cycle, as well as when free running (in complete darkness). This research is essential for understanding broader mechanisms of circadian dysregulation in neurodegeneration. It could provide a foundation for exploring clock genes as therapeutic targets to mitigate activity, sleep, and circadian rhythm disturbances in FTD patients.

522S **Probing autophagic flux in metastatic cells in the *Drosophila* wing disc** Luz M Arvizu[1], Jeslin Jacob[2], Kruthi Kumar[2], Yadanar Khin[2], James Tower[2], Aaliyah Molina[2], Danino Corsis[2] [1]Biological Sciences, San Jose State University, [2]San Jose State University

Cancer research has traditionally focused on how mutations and dysregulated signaling pathways contribute to tumorigenesis; however, much less is known about how changes in cellular environments, including intracellular pH (pHi), regulate cancer cell behaviors such as metastasis and autophagy. Increased pHi is a conserved feature of cancers regardless of genetic mutations or tissue of origin, and is sufficient for hyperproliferation, tissue dysplasia and increased metastatic invasion. Metastatic cancer is the primary cause of death for more than 90% of patients with malignant solid tumors. We recently showed that increased pHi is sufficient to induce the catabolic autophagy pathway, which is implicated in metastasis, and is thought to facilitate cancer progression. Autophagy is a conserved process that allows for degradation or recycling of cellular building blocks through the lysosome. In cancer cells, autophagy is upregulated to help tumor cells survive nutrient deprivation and harsh environmental conditions. Our lab developed tools to increase pHi in the absence of other transforming mutations through overexpression of sodium-proton exchanger, *DNhe2* (homolog of mammalian NHE1). We showed that over-expression of *DNhe2* increases pHi in the developing *Drosophila* eye and wing imaginal discs. We used the *patched-GAL4* driver to express oncogenic *RasV12* in a stripe of cells in the wing imaginal disc and found that co-expression of *DNhe2* induces metastatic behaviors, including basal expansion, single cell invasion, and invasive streaming of cells. Our current objective is to determine how autophagic flux changes in metastatic tumors. We will induce tumors through expression of *RasV12* and measure autophagy markers. We will label lysosomes using Lysotracker, and also evaluate autophagy markers using fluorescently-tagged proteins like Atg8A and p62 in *RasV12*-induced tumors, and compare fluorescence intensity to control tissues. We predict that metastatic cells will display higher levels of autophagy due to increased pHi and that this contributes to metastatic invasion. Data from these experiments will link dysregulated tumor pHi to altered cellular metabolism and pathogenic invasive cell behaviors.

523S **Characterization of Drosophila Amyotrophic Lateral Sclerosis (ALS) Upon Genetic Modification to Stress Granule-Associated Genes** Emily Sarkisian[1], Sarah Twinney[1], Anqi Zhou[2], Kristi A. Wharton[2] [1]Biotechnology Graduate Program, Division of Biology and Medicine, Brown University, [2]Department of Molecular Biology, Cell Biology, and Biochemistry, Brown University

Stress granules (SG) are membraneless, ribonucleoprotein structures that assemble as a protective response to stress in the cell. In the presence of chronic stress, SGs fail to dissolve, leading to persistent aggregation of proteins and stalled mRNA translation. SG dysfunction is a hallmark of many neurodegenerative diseases, including amyotrophic lateral sclerosis/frontotemporal dementia (ALS/FTD), yet it is unclear whether SG dysfunction is causative to these disorders or is in response to neurodegeneration.

Mutations to the genes *C9orf72* and *TARDBP* are seen in both sporadic and familial forms of ALS/FTD. We developed an adult-onset Drosophila model of *C9orf72-(G4C2)49* ALS, wherein adult flies exhibit a shortened lifespan and exacerbated decrease in climbing velocity with age. Using this and a motor neuron-driven dTARDBP patient allele (*tbph[N493D]*), we tested if knocking down four SG-associated genes of interest using RNAi had any effect on neurodegeneration phenotypes in these two models. These genes (Heterogeneous nuclear ribonucleoprotein at 27C (*Hrb27C*), Tudor staphylococcal nuclease (*Tudor-SN*), maleless (*mle*), and ovarian tumor (*otu*)) were selected from a screen that identified ~150 novel human SG components (Markmiller et al., 2018). As shown previously, RNAi knockdown of each gene reduces the rough eye phenotype associated with GMR>hTDP-43[M337V] and hFUS[R521C] in Drosophila. To better understand each of these four SG genes and their role in alleviating neurotoxicity, we assessed the integrity of the NMJ (bouton number), viability (eclosion), motor function (climbing), and lifespan with knockdown of each gene. The suppression of neurotoxicity associated with *C9orf72-(G4C2)49* ALS and *tbph[N493D]* ALS models varied with each gene knockdown. Our analysis highlights the dynamic role of SG-associated genes in ALS pathology.

524S **Effects of *parkin* and *rosy* on Mortality, Motor Dysfunction, and Malpighian Tubule Stone Formation in Parkinson's Disease Model *Drosophila*** Aaron McMurray, Ben Doan, Rashmi Sethi, Gerald Call, Krista Pearman Midwestern University

Parkinson's Disease (PD) is a prevalent neurological disorder characterized by progressive motor dysfunction, neurodegeneration, and early mortality, which currently has no cure. One of the most commonly used *Drosophila* models of PD is the *park*^{25} mutant (with >65 references on FlyBase) which has a loss-of-function mutation in *parkin (park)*, an E3 ubiquitin ligase associated in humans with the less common, early-onset form of PD. Mutant *park* flies exhibit motor deficits, reduced lifespan, altered microbiome, mitochondrial dysfunction and neurodegeneration, comparable to human PD patients. We recently found that homozygous *park*^{25} flies also develop stones in the Malphigian tubule (i.e., "kidney stones") but identified that this phenotype was due to another mutation on the same chromosome in the *rosy* (*ry*) gene. *Ry* encodes an enzyme that converts xanthine to uric acid (orthologous to human *XDH* [xanthine dehydrogenase]), consistent with recently established metabolomic xanthine disruption in humans. This study was performed to analyze phenotypes identified in the *park*^{25} fly in order to determine which phenotypes were due to mutations in *park* or *ry*, or potentially a synergistic action with both. These experiments utilized >5,000 flies from 12 different genotypes with various allelic combinations of *park* and *ry* to demonstrate their involvement in five relevant phenotypes: eclosion, lifespan, motor function via climbing and flight assays, and development of Malphigian tubule stones. Mutation in *park* cause decreased lifespan, climbing, and flight phenotypes, reinforcing previous data. However, only flies with both *park* and *ry* mutations exhibit decreased eclosion rates and stone formation, indicating that a synergistic action by both genes may be producing these phenotypes. Importantly, lifespan and eclosion rates in the homozygous *park*^{25} flies were significantly more reduced than all other flies. This prompted us to perform programmatic analysis of the combined data, which suggests a previously unidentified additional mutation on the *park*^{25} chromosome, which appears to possibly contribute to all five of the phenotypes. The identity of this unknown mutation is currently being investigated via deficiency screen.

525S **Effects of Restricted a-Synuclein Expression in Subsets of Dopamine Neurons on Neurodegeneration and Locomotion** Trisha Gongalore[1], Chisato Kamakura[2], Sandra Watson[3] [1]Pomona College, [2]Claremont McKenna College, [3]The Department of Natural Sciences at Pitzer and Scripps Colleges

Parkinson's disease (PD) affects an estimated 10 million people worldwide and is characterized by motor deficits and the progressive loss of dopaminergic (DA) neurons in the substantia nigra pars compacta (SNpc). While current research has shown that both environmental and genetic factors are likely contributors of PD, the cause of DA neuron loss and why some neurons are more susceptible to degeneration remains elusive. Aggregates of the protein α-synuclein (α-syn), which is encoded by the gene SNCA in humans, are a hallmark of PD in degenerated cells. The plethora of established genetic, imaging, and behavioral tools in Drosophila make it a powerful model system to investigate how expression of SNCA in a subset of neurons can impact degeneration of neurons and glia in other areas of the brain. We aim to determine whether toxic overexpression of α-syn in targeted neuron pools can induce degeneration in other DA neuron populations by restricting the expression of SNCA variants (wild-type and mutant A53T, which are used to model PD in flies) to specific subsets of DA neurons. Using existing split-GAL4 lines to drive these transgenes, we will assess neuron counts in the anterior and posterior DA clusters and surrounding glia and investigate potential age-dependent motor decline. Concurrently, we will use the Drosophila Activity Monitor System to understand how restricted expression of α-syn impacts sleep, activity, and overall survival. Our preliminary results show differences in survival when expressing α-syn in different subsets of DAergic neurons. Additionally, our initial findings show a minimal decrease in activity after 7 days in flies expressing α-syn under various drivers. Further investigation will follow-up on these preliminary findings to determine if differences are due to α-syn expression. Continued study will determine the pattern of neurodegeneration with age in DA neurons and glia using the split-GAL4s. These studies will provide critical insight into how disrupted protein homeostasis in a subset of neurons can influence overall neuronal homeostasis, provide insight into variations in neuronal vulnerability to degeneration, and add to our knowledge on the initiation and subsequent cascade of degeneration seen in PD.

526S **Transcriptomic analysis identifies muscle-specific mitochondrial and vesicular transport genes as methylmercury toxicity targets in a Drosophila model of Congenital Minamata Disease** Catherine R Beamish[1], Jennifer D Becker[2], Lok Ming D Tam[1], Tanzy D Love[3], Matthew D Rand[1] [1]Environmental Medicine, University of Rochester, [2]Genomics Center, University of Rochester, [3]Biostatistics, University of Rochester

Methylmercury (MeHg) is a ubiquitous and potent environmental contaminant that bioaccumulates in fish and seafood. Congenital Minamata Disease (CMD) is a condition resulting from high level fetal MeHg exposure, where offspring present with cognitive and motor deficits, much like cerebral palsy. MeHg is a canonical developmental neurotoxicant. Skeletal muscle as a post-synaptic MeHg target and contributor to CMD has garnered far less attention. Using *Drosophila* to model CMD we have shown that MeHg exposure in the larval/pupal stages can elicit graded and latent dose responses affecting adult longevity and flight behavior at lower doses (0.4-2.5 ppm in food) and eclosion and pupariation at higher doses (>2.5 ppm in food). The latter are accompanied by dysmorphogenesis of indirect flight muscles (IFMs). To distinguish muscle versus neural effects of MeHg we have implemented a 2 X 2 X 2 strategy using two MeHg treatments (0µM and 5µM in food); two tissues (IFM and ventral nerve cord (VNC)); two developmental life stages (pupa and adult). First instar larvae reared on food with or without MeHg were collected as pupae (30 hours APF) or adults. Upon eclosion, adult flies were transferred to MeHg-free food and harvested on day 15. RNA-seq analysis was performed on transcripts from isolated indirect flight muscle (IFM) and ventral nerve cord (VNC). Differential expression (DEseq) and clustering analysis (MFuzz) of transcript levels was combined to resolve gene sets for Gene Ontology (GO) analysis (GOrilla). MeHg exposure produced 10-times more DE transcripts in the IFM compared to the VNC. Among known MeHg response genes, Nrf2 antioxidant response pathway genes showed muscle-specific MeHg-induced expression changes. The Activity-regulated cytoskeleton protein 1 (Arc1, *CG12505*), an early response gene and regulator of synaptic plasticity, was upregulated by MeHg in both tissues at both life stages. In the IFM transcriptome, the most enriched and significant GO terms identified were 'Mitochondrial ribosomal translation' at the pupa stage and 'Mitochondrial respiratory chain complex I' and 'ESCRT III complex' in adults, all showing decreased expression with MeHg. Our findings uncover a novel muscle-specific role for mitochondria and intercellular vesicular communication mechanisms as targets in MeHg toxicity and the etiology of CMD. The findings also support the hypothesis that developing skeletal muscle is highly susceptible to MeHg.

527S **The role of inflated in aging-associated impulsivity and loss of memory** Ali P Ballesteros, Kyung-An Han, Paul Sabandal The University of Texas El Paso

Dementia is characterized by progressive decline in cognitive functions such as memory and inhibitory control. The most common dementia Alzheimer's disease (AD) is caused by genetic and non-genetic risk factors such as aging and social stress; however, whether and how they interact for AD is poorly understood. To fill this knowledge gap, we conducted a functional genetic screen and identified inflated (if) which codes for alpha-integrin, a cell adhesion molecule located in the plasma membrane and involved in many developmental processes. We found that the heterozygous if mutants (if/+) exhibited impulsivity and memory loss in an aging-dependent manner. We also found that the MB is the functional site for if since if knockdown in the mushroom body (MB) neurons triggered aging-dependent impulsivity. The goal of this study is to determine the mechanism by which if causes aging-related cognitive decline. For the task, we first investigated the MB synaptic integrity by immunohistochemical analyses of synaptic molecules including Fas2, DLG and BRP. At 4 days old (young age) no difference was observed in the MB Fas2 immunoreactivity in if/+ and CS. At 4 and 6 weeks old (early old and old ages, respectively), however, if/+ exhibited altered as shorter and split MB lobes. We are currently examining the presynaptic Bruchpilot and postsynaptic DLG. Our study will provide novel insights into the role of alpha-integrin in dementia.

528S **Exploring the role of Sirt6 in the *drosophila melanogaster* in neurodegeneration and the healthy aging brain** Samira Xhaferi[1], Prema Singaravel[1], Roja Sharma[1], Chelsea Jesmer[1], Neelanjana Roy[1], Masashi Tabuchi[2], Jackson Taylor[1] [1]Center for Gene Regulation in Health and Disease, Department of Biological, Geological and Environmental Sciences, Cleveland State University, [2]Case Western University

Sirt6, a member of the Sirtuin family of NAD^+ dependent deacetylases, is a multifunctional enzyme which plays key roles in gene regulation, DNA repair, and metabolism. In mice and flies, overexpressing Sirt6 extends lifespan, suggesting a conserved role as an anti-aging gene. Advanced age is the leading risk factor for many neurodegenerative diseases, including Alzheimer's Disease. Sirt6 mRNA and protein levels are reduced Alzheimer›s patients and experimental reduction of Sirt6 levels in animal and cell culture models leads to neurodegenerative phenotypes and increased phosphorylation and acetylation of Tau protein, modifications associated with pathological effects. Together, these data suggest an important neuroprotective role of Sirt6 in Alzheimer's disease. In the present study, we utilized *Drosophila melanogaster* as a model system to explore the role of Sirt6 in Alzheimer›s disease and other tauopathies, alongside investigating its role in the healthy aging brain. We found that *Sirt6* mRNA levels were significantly reduced in the heads of flies with neuronal expression of the 0N4R variant of human Tau (hTau) protein, consistent with observations made in Alzheimer›s patients. Notably, overexpressing Sirt6 rescued learning defects caused by neuronal hTau expression. Sirt6 overexpression also rescued defects in both climbing ability and shortened lifespan found in flies expressing hTau R406W, a mutant form of Tau associated with frontotemporal dementia. Moreover, we found that Tau R406W expression in the nervous system led to increased retrotransposon expression, and that Sirt6 overexpression prevents this increase. We also examined the effect of reducing Sirt6 levels on normal brain function. We found that flies with genetic deletion of Sirt6 or targeted knockdown in specific circadian neurons have dysregulated sleep profile. Sirt6 deletion flies also exhibited increased levels of γ-H2AV, a marker of DNA double-strand breaks, in the optic lobe compared to age-matched control flies. Collectively, these results provide evidence of Sirt6›s neuroprotective role against Tau-induced neurotoxicity and its central role in the biology of the aging brain.

529S **A drug repurposing screen identifies N-acetyl-L-leucine as a candidate therapeutic for SYNGAP1-related disorder** Haley Tokars, Katherine Beebe, Emily Coelho, Caroline Massey, Heather Evans, Clement Chow Human Genetics, University of Utah

Single gene mutations of SYNGAP1 account for about 1% of all intellectual disabilities. SYNGAP1-related disorder is a rare neurodevelopmental disorder characterized by loss of function mutations in the *SYNGAP1* gene, which result in symptoms including cognitive impairments, developmental delays, and epilepsy. There is no cure, and the current treatment options only address symptom management. We completed a drug repurposing screen in a *Drosophila* model of SYNGAP1. With an eye-based phenotype, we screened 1520 compounds from the Prestwick Chemical Library and identified several promising hits. Further validation revealed that N-acetyl-L-leucine (NALL) had the strongest validation data. NALL is a modified acetylated enantiomer of the amino acid leucine, which has been approved as a treatment for vertigo, under the name Tanginil, in France for at least 60 years. We will present tissue-specific analyses that will characterize which tissues NALL is most effective, with attention towards neurons and glia. We will also present transcriptomic data in treated and untreated SYNGAP1 model *Drosophila* to shed light on the mechanism of action of this rescue. This work suggests that NALL is a potential new therapeutic compound for SYNGAP1 and demonstrates a novel role of leucine in SYNGAP1 dysfunction.

530S **The role of the synaptic adhesion molecule *Kekkon5* in Alzheimer›s disease and related dementias** Kryssia Villarreal, Paul R Sabandal, Kyung-An Han Biological Sciences, University of Texas at El Paso

Alzheimer's disease and related dementias (ADRD) currently affect approximately 6 million adults with steady increase in prevalence in the U.S. ADRD symptoms include impulsivity and memory loss, which are exacerbated by non-genetic risk factors such as aging and social stress. Genetic and non-genetic risk factors for ADRD have been studied; however, whether and how these factors interact for ADRD is understudied. We performed an unbiased genetic screen in *Drosophila melanogaster* for novel impulsivity genes and uncovered the synaptic adhesion molecule *Kekkon5* (*Kek5*). Specifically, the *Kek5* heterozygous flies (*Kek5/+*) exhibited impulsivity in an aging-dependent manner in the Go/No-Go test. In addition, the *Kek5/+*'s impulsivity phenotype is sensitive to social stress, where the aged *Kek5/+* tested in a group showed high impulsivity while the aged *Kek5/+* tested alone showed robust inhibitory control. Together, these data indicate that individual risk factors either *Kek5* deficiency, aging, or social stress are not sufficient to cause impulsivity, but the interaction of all the risk factors noted above is required for impulsivity. This study is directed to identify the mechanism by which *Kek5* interacts with the non-genetic risk factors aging and social stress for ADRD. *Kek5* is expressed in the mushroom body (MB) a/b neurons, and we have previously identified that the MB neurons are important for inhibitory control. To examine whether *Kek5* deficiency affects the MB synaptic integrity, we conducted immunohistochemical analyses for synaptic molecules including Fas2, DLG, and BRP across different ages (4 days – young age, 2 weeks – middle age, 4 weeks – early old age, and 6 weeks – advanced old age). We found that across all ages tested the Fas2 expression pattern in the MB axons was not altered; however, the 2- and 4-week-old *Kek5/+* MB had significantly reduced Fas2 expression. This suggests that the *Kek5* may not be crucial for the MB development but important for the MB function in inhibitory control upon interacting with Fas2. We are currently investigating how *Kek5* interacts with Fas2 on synaptic integrity as well as impulsivity, learning, and memory. Our study will provide novel insights into the mechanism by which synaptic adhesion molecules like *Kek5* and Fas2 functionally contribute to ADRD.

531S ***Inflated*** **Interacts with Hyper Dopamine to Cause Impulsivity** Veronica C Ciliberto, Paul R Sabandal, Kyung-An Han Biology, University of Texas at El Paso

Impulsivity is associated with autism spectrum disorder (ASD) and attention-deficit/hyperactivity disorder (ADHD). It is influenced by genetic factors such as dopamine (DA) transporter and receptor polymorphisms; however, the mechanism by which DA signaling affects impulsivity is poorly understood. To narrow this knowledge gap, we conducted an unbiased genetic screen and identified *inflated* (*if*) as a novel impulsivity gene. *if* encodes one of the five integrin alpha subunits. Inflated is involved in cell growth, survival, and proliferation as well as axon growth and synaptic plasticity. It is expressed in the brain including the mushroom body (MB) neurons, which is a key neural site for inhibitory control, learning and memory. Here, we sought to identify the mechanism by which the *if* x *fumin* (*fmn*) interaction causes impulsivity. We first examined whether *if* alters DA neuronal integrity by conducting immunohistochemical analysis on tyrosine hydroxylase (TH), the rate-limiting enzyme in DA biosynthesis. The $if^3/+$ brains displayed substantially reduced TH immunoreactivity (IR) compared to the wild-type *Canton-S* (*CS*). Specifically, the most significant decrease in TH IR in the $if^3/+$ was in the DA axons projecting to the MB. We thus examined the MB synaptic integrity by immunostaining for DLG (discs large; a post-synaptic molecule). Compared to *CS*, we found increased DLG IR in the $if^3/+$ MB especially in the alpha3 and alpha'3. Together, these findings point to altered DA-MB synapses as a potential mechanism by which *if* causes impulsivity. We are currently investigating additional synaptic molecules, learning and memory in the $if^3/+;fmn/+$ double as well as single heterozygous mutants. Our study will provide new insights on how Integrins contribute to impulsivity that may have clinical implications for ASD and ADHD.

532S **The Human Antimicrobial Peptide, LL-37, Diminishes Aβ42's Effects on Longevity, Behavior, and Gene Expression in a Drosophila Model of Alzheimer's Disease** Miles Maybrun[1], Julia Aguiar[1], Elizabeth Castillo Nava[1], Jules Rivera[1], Aranza Gomez[1], Noah Evans[1], Chike Udemezue[1], MaiLan Kasch[1], Belal Alatasi[1], Kenneth Owyang[1], Allison Swick[1], Kayla Azad[1], Waleed Alsibai[1], Jeremy Lee[1], Annelise Barron[2] [1]University of California, Santa Cruz, [2]Stanford

The amyloidogenic peptide Aβ42 is thought to play a major role in Alzheimer's Disease (AD), specifically related to its tendency to form neurotoxic aggregates. In vitro, the human antimicrobial peptide LL-37 has been shown to decrease the formation of Aβ42 amyloid fibrils (De Lorenzi, et al., 2017), suggesting that LL-37 and its interaction with Aβ42 might reduce the deleterious effects of Aβ42 on neurons.

To test this hypothesis, four transgenic Drosophila lines were generated and used to test whether LL-37 expression alleviates Aβ42's negative effects on flies: flies pan-neuronally expressing Aβ42 alone, expressing LL-37 alone, co-expressing Aβ42 and LL-37, and non-expressing controls. These flies were used in longevity assays and RNA sequencing. Co-expressing LL-37 along with Aβ42 showed a significant rescue in longevity compared to flies solely expressing Aβ42, both in survivorship to eclosion as well as adult survival. Interestingly, expressing LL-37 on its own shows a detrimental effect on both survivorship to eclosion and adult survival. RNA sequence profiling shows that LL-37 reverses changes in gene expression caused by Aβ42, specifically for genes relating to proteolysis. To better understand how LL-37 is actually mitigating AD pathology caused by Aβ42, we are performing rapid iterative negative geotaxis (RING) assays, since Aβ42 is known to have deleterious effects on climbing, which become more severe as the flies age. Additionally, AD is intrinsically connected to sleep, so a sleep assay will allow us to observe if LL-37 can mitigate sleep related symptoms caused by Aβ42.

In order to determine whether Aβ42 and LL-37 interact in vivo, we are performing co-immunoprecipitation and immunohistochemistry in co-expressing flies. To further cement and visualize LL-37s influence on AD pathology, we are using scanning electron microscopy of adult eyes and immunohistochemistry of eye imaginal discs to analyze whether LL-37 expression mitigates apoptosis in eye discs and abnormalities within eyes we have observed in flies expressing Aβ42 under the GMR-GAL4 driver.

533S **Identification of a conserved functional motif in the Huntington's Disease-associated HTT-HAP40 core complex** Stephen Farmer[1], Amanda Solbach[1], Shiyu Xu[1], Beatriz Rios[1], Xin Ye[1], Amy Gao[2], Daniela Covarrubias[3], Yue Yu[1], Lili Ye[1], Erin Furr Stimming[4], Sheng Zhang[1] [1]Center for Metabolic and Degenerative Diseases, University of Texas Health Science Center at Houston, [2]University of Texas at Austin, [3]Rice University, [4]McGovern Medical School, University of Texas Health Science Center at Houston

Huntington's disease (HD) is a neurodegenerative disorder caused by an abnormal expansion of CAG repeats in the Huntingtin (HTT) gene. Despite its simple genetic etiology, HD's complex pathogenic mechanisms have driven interest in targeting HTT for treatment, underscoring the need for a comprehensive understanding of HTT regulation. HTT primarily forms a structured core complex with HAP40, comprising two large globular domains connected by a bridge domain. Our previous work demonstrated that HAP40, conserved in Drosophila, regulates HTT's function, stability, and levels, suggesting it may play a significant role in modifying HD pathogenesis. Here, we show that HTT and HAP40 synergize to produce novel gain-of-function effects in Drosophila when overexpressed. Protein modeling revealed that despite extensive evolutionary and sequence divergence, the HTT-HAP40 complexes in flies and humans exhibit a high degree of structural similarity. Protein-contact mapping and coarse-grained molecular dynamics indicate that HAP40 preferentially binds to HTT's C-terminal domain in both species. Structural analysis of the HTT-HAP40 interface identified ten conserved interactions essential for HAP40's binding to HTT, validated through follow-up mutational studies in human cells. Furthermore, we identified a conserved, solvent-exposed N-terminal motif on HAP40, termed BΦ, that does not directly bind HTT. Mutational studies in flies and human cells revealed that while HAP40's BΦ motif is not essential for HTT binding or stability, it is crucial for the core complex's function. These structural-functional analyses suggest that structural similarity underlies functional conservation across species, providing new insights into HAP40's regulatory role and interaction with HTT and highlighting a potential therapeutic target for HD.

534S **Dysfunctional BCAA degradation triggers neuronal damage through disrupted AMPK-mitochondrial axis due to enhanced PP2Ac interaction** Chun-Hong Chen[1], Shih-Cheng Wu[2] [1]Institute of Infectious diseases and Vaccinology, NHRI, [2]Department of Clinical Laboratory Sciences and Medical Biotechnology, National Taiwan University

Metabolic and neurological disorders commonly display dysfunctional branched-chain amino acid (BCAA) metabolism, though it is poorly understood how this leads to neurological damage. We investigated this by generating *Drosophila* mutants lacking BCAA-catabolic activity, resulting in elevated BCAA levels and neurological dysfunction, mimicking disease-relevant symptoms. Our findings reveal a reduction in neuronal AMP-activated protein kinase (AMPK) activity, which disrupts autophagy in mutant brain tissues, linking BCAA imbalance to brain dysfunction. Mechanistically, we show that excess BCAA-induced mitochondrial reactive oxygen species (ROS) triggered the binding of protein phosphatase 2A catalytic subunit (PP2Ac) to AMPK, suppressing AMPK activity. This initiated a dysregulated feedback loop of AMPK-mitochondrial interactions, exacerbating mitochondrial dysfunction and oxidative neuronal damage. Our study identifies BCAA imbalance as a critical driver of neuronal damage through AMPK suppression and autophagy dysfunction, offering insights into metabolic-neuronal interactions in neurological diseases and potential therapeutic targets for BCAA-related neurological conditions.

535S **Impact of metal exposure on lifespan in *drosophila melanogaster*** Gelila Isayas, Fernando Vonhoff Department of Biological Sciences, University of Maryland, Baltimore County

Drosophila melanogaster (or also known as fruit fly) is a useful model organism due to its short life cycle, conserved genome, and various genetic tools. Fruit flies share roughly 13,000 protein coding genes with high homology to human disease-related genes. This project focuses on studying the fly homolog of Amyloid Precursor Protein (APP). APP is an integral membrane protein, encoded by the APP gene on chromosome 21 in humans, and is strongly implicated in the pathogenesis of Alzheimer's Disease (AD). Interestingly, recent studies report the link between AD and metal exposure in humans. APP is highly conserved across species, with Amyloid Precursor Protein-Like (APPL) serving as its homolog in fruit flies. Metal homeostasis in fruit flies is crucial for maintaining cellular function with dysregulation and metal-induced toxicity negatively impacting survival and physiological performance. This study investigates the impact of metals such as copper and aluminum, on the lifespan of *Drosophila melanogaster*. A survival assay was performed with wild-type flies (WT) and APPL loss-of-function mutant flies (APPL-d), which were subjected to various metal concentrations based on previous literature. Fly mortality was recorded on specific days following metal exposure. Preliminary results suggest that high concentrations of aluminum shortens the lifespan of APPL-d flies more than WT flies but WT flies are more vulnerable to high copper concentrations than APPL-d flies. Overall, this research will help us better understand the fly homolog APPL and the effects of metal exposure in fly survival, with the ultimate goal of translating these findings to the study of APP in humans.

536S **From Bugs to Biomarkers, Investigating Chitinase-Like Proteins in Tube Morphogenesis** Richelle Chen, Luana Paleologu, Celeste Berg University of Washington

Chitin is a structural polysaccharide composed of modified monomers of glucose; it is a key component of the exoskeletons of arthropods, crustaceans, and insects. Synthesized at over one billion tons each year, chitin is the second most abundant polysaccharide in nature. While mammals do not synthesize chitin, they do synthesize chitinases and enzymatically-inactive chitinase-like proteins (CLPs). In humans, CLPs serve as biomarkers for a variety of diseases, including tumor metastasis, schizophrenia, asthma, and other inflammatory conditions. Nonetheless, there is little knowledge of their function or how their expression is regulated.

We identified several orthologs of human CLPs—members of the *Drosophila* family of Imaginal disc growth factors (Idgfs)—in a proteomics analysis designed to discover regulators of dorsal-appendage tube morphogenesis in the fly ovary [Zimmerman et al. 2017]. Comparative mass spectrometry revealed an increase of all six Idgfs as a downstream consequence of a loss of function in the SOX transcription factor Bullwinkle (*bwk*). Overexpression of Idgf1 or Idgf3 produced large open tubes due to a failure in epithelial zippering and to aberrant cell migration, with Idgf3 exerting a stronger phenotypic effect than Idgf1. A sextuple mutant lacking all Idgfs produces eggs with no dorsal appendages [Sustar et al. 2023], underscoring the essential role of these proteins.

To investigate whether the differential effects of the Idgfs are due to differences in protein homology or variable expression resulting from random genomic insertion of the over-expression transgenes, we designed a controlled experimental system utilizing the *GAL4-UAS* system. We created all six *UAS-Idgf* constructs and integrated them into the same *attP* site in the genome to ensure uniform genomic context. Using a variety of Gal4 drivers, we will present data comparing expression levels and phenotypic outcomes on dorsal appendage formation. We hypothesize that the differential effects of the Idgfs are due to differences in protein homology. Of the single-gene knock outs, only *Idgf6* produced a phenotype; we therefore speculate that Idgf6 overexpression will be most impactful on tube formation.

This study provides a framework to disentangle the specific roles of Idgf family members in *Drosophila* development and better understand their human orthologs in tissue remodeling, inflammation, and tumor metastasis.

537S **A *Drosophila* model for Uveal Melanoma: identifying genes with a functional role in oncogenic *GNAQ* phenotypes** Emaan Tehseen[1], Cathie M Pfleger[2], Anne M Bowcock[2], Max Luf[3], Priya Begani[2], Felix Rosemann[2] [1]Department of Oncological Sciences, The Icahn School of Medicine at Mount Sinai, [2]Department of Oncological Sciences, The Icahn School of Medicine at Mount Sinai, The Tisch Cancer Institute, The Graduate School of Biomedical Sciences, [3]Department of Oncological Sciences, The Icahn School of Medicine at Mount Sinai, The Tisch Cancer Institute

Uveal melanoma (UM) is the most common cancer of the eye with a low survival rate due to its high metastatic risk. UM develops in melanocytes within the uvea following an initial mutation primarily in *GNAQ/11*, such as oncogenic mutation $GNAQ^{Q209P}$, followed by a secondary mutation, such as in *SF3B1* or loss of *BAP1*. There is no established cure for UM with existing treatments such as enucleation and radiation therapy being aggressive and conferring poor quality of life. *Drosophila melanogaster* possesses orthologs of the genes mutated in UM, and their eye imaginal disc tissue is a monolayer epithelial tissue well established as a model system to study epithelial cancers like UM. This study utilized *Drosophila* to model oncogenic *GNAQ* signaling by using GMR-gal4 to drive expression of $GNAQ^{Q209P}$ in the late developing fly eye. Using this context, we investigated a functional role for genes involved in UM, genes downstream of *GNAQ* signaling, or genes suggested as UM therapeutic targets through genetic interactions by driving concurrent RNAi or introducing loss of function alleles in candidate genes. Genetic interactions with RNAi lines for *IP3R* (downstream of *GNAQ*) and *HDAC* (a candidate therapeutic target in UM) showed dramatic suppression of oncogenic phenotypes, suggesting that these genes are functionally relevant to $GNAQ^{Q209P}$ phenotypes. We also identified other candidates whose knockdown suppressed the $GNAQ^{Q209P}$ eye phenotypes. Future research can utilize this model to characterize additional genes for their functional relevance to UM, to characterize how secondary mutations in *SF3B1* or *BAP1* lead to malignancy, and to screen FDA-approved drugs to identify therapeutics with potential to treat this deadly disease.

538S **Prophylactically feeding manganese to *Drosophila* confers sex-specific protection from acute ionizing radiation independent of MnSOD2 levels.** Robert P Volpe[1,2], Aditya Sen[1,2], Ajay Sharma[3], Venkatesan Kathiresan[3], Brian M Hoffman[3], Rachel T Cox[4] [1]Biochemistry and Molecular Biology, Uniformed Services University, [2]Henry M Jackson Foundation for the Advancement of Military Medicine, [3]Chemistry, Northwestern University, [4]Uniformed Services University

Ionizing radiation causes severe and intractable injury. Despite over a century of research, no FDA-approved prophylactic countermeasures for radiation injury currently exist. Ionizing radiation indiscriminately damages molecular bonds in all biomolecules and generates cytotoxic levels of reactive oxygen species through the radiolytic decomposition of water molecules. Studies of radiation resistance in extremophilic organisms have revealed mechanisms of protection against ionizing radiation via the hyperaccumulation of manganous ions (Mn^{2+}), which spontaneously complex with orthophosphate and metabolites to form high-symmetry, small-molecule, non-enzymatic antioxidant complexes (H-Mn) that efficiently scavenge reactive oxygen species. Using the Drosophila model, we examine the radioprophylactic potential of oral Mn^{2+} supplementation in the form of manganese chloride ($MnCl_2$) to improve survival following high-dose gamma radiation exposure. Our findings reveal an innate gender-associated difference in radiation survival: male flies are significantly more radiosensitive than females. Pre-exposure treatment with dietary $MnCl_2$ at micromolar concentrations increases radiation survival in the more sensitive males but not in females. Western blot analysis indicates that $MnCl_2$ treatment does not influence MnSOD enzyme expression, while electron paramagnetic resonance spectroscopy demonstrates increased levels of non-enzymatic Mn-phosphate antioxidant complexes with treatment. These findings suggest that dietary manganese supplementation may serve as an effective prophylactic radiation countermeasure and invite further inquiry into both gender-driven differences in radiosensitivity and site-specific radioprotection from $MnCl_2$ intervention.

539T **Establishing Drosophila as a model for sleep disturbances related to FASD** Reza Almassi[1], Monica Flores Tapia[1], Navneet Sanghera[1], Rachael French[2] [1]Biological Sciences, San Jose State University, [2]San Jose State University

Developmental alcohol exposure (DAE) causes neurological defects in both mammals and Drosophila. In humans, fetuses exposed to alcohol may eventually develop Fetal Alcohol Spectrum Disorder (FASD) resulting in various effects including reduced brain size, low body weight, intellectual disabilities, behavioral changes and increased sleep disturbances. Previous studies have shown that Drosophila exposed to alcohol during their development display similar effects such as impaired motor coordination, abnormal neural development, and shortened lifespan. However, the effects of ethanol exposure on circadian rhythm and sleep disorders in Drosophila has not been investigated to a large extent. We hypothesize that Drosophila exposed to ethanol will exhibit sleep disturbances, similar to those experienced by humans with FASD. To test this hypothesis, we will perform a locomotion assay using the Drosophila DAM5H activity monitoring system to assess the movement patterns of wild-type flies reared on ethanol versus control media. Once the protocols for this system are established, we will expand our research to different Drosophila mutants. We will test mutant flies' sensitivity to DAE by assessing sleep disturbances and nocturnal wakefulness. Mutants of interest could include Cry, fmn, and Sss, each of which represent distinct pathways involved in sleep and circadian regulation.

540T **Contribution of neurons that express *fruitless* and *Clock* transcription factors to behavioral rhythms and courtship** Anthony Deluca[1], Brooke Bascom[1,2], Matthew Kocher[1], Daniela Planas[1], Marielise N Torres[1], Michelle N Arbeitman[1] [1]Biomedical Sciences, Florida State Univ, [2]Florida State University

Animals need to integrate information across neuronal networks that direct reproductive behaviors and circadian rhythms. In Drosophila, the master regulatory transcription factors that direct courtship behaviors and circadian rhythms are co-expressed in a small set of neurons. In this study we investigate the role of these neurons in both males and females. We find sex-differences in the number of these *fruitless* and *Clock* -expressing neurons (*fru Ω Clk* neurons) that is regulated by male-specific Fru. We assign the *fru Ω Clk* neurons to the electron microscopy connectome that provides high resolution structural information. We also discover sex-differences in the number of *fru*-expressing neurons that are post-synaptic targets of *Clk*-expressing neurons, with more post-synaptic targets in males. When *fru Ω Clk* neurons are activated or silenced, males have a shorter period length. Activation of *fru Ω Clk* neurons also changes the rate a courtship behavior is performed. We find that activation and silencing *fru Ω Clk* neurons impacts the molecular clock in the sLNv master pacemaker neurons, in a cell-nonautonomous manner. These results reveal how neurons that subserve the two processes, reproduction and circadian rhythms, can impact behavioral outcomes in a sex-specific manner.

541T **Rhythms in structural plasticity of clock neurons are required for circadian behavior** Sukthi Gunda[1], Rebecca Simen[1], Andreas Jenny[2], Justin Blau[1] [1]Biology, New York University, [2]Developmental & Molecular Biology, Albert Einstein College of Medicine

The small LNv (s-LNv) circadian pacemaker neurons set the pace of *Drosophila* circadian behavioral rhythms in constant darkness. s-LNv projections show rhythms in structural plasticity, making and breaking synaptic connections with downstream neurons in the dorsal brain with a 24 hour rhythm. However, the precise role of this plasticity has been challenging to address.

Puratrophin-1-like (*Pura*) is a key regulator of s-LNv plasticity rhythms. *Pura* is rhythmically expressed and encodes a Rho1 GEF. *Pura* RNA rhythms lead to rhythms in Rho1 activity in s-LNv projections that peak around dusk to retract s-LNv projections and break connections with downstream neurons.

We have analyzed two novel *Pura* null alleles generated by CRISPR. We found that s-LNv projections are in a constitutively expanded state in the absence of *Pura*. Thus *Pura* is essential for s-LNv projections to retract. We also found that the behavioral rhythms of *Pura* mutant flies are ~2 hour longer than normal flies. We also see period-lengthening when somatic CRISPR is used to remove *Pura* only from LNvs. We propose that these long periods arise from s-LNvs being over-connected with downstream neurons in the absence of *Pura* and thus of Rho1 activity. Our data reinforce the idea that 24 hour behavioral rhythms are a property of the circadian neuronal network, and indicate that circadian behavior requires structural plasticity rhythms.

542T **Multiple mechanisms of action for an extremely painful venom** Lydia J Borjon[1,2], Luana C de Assis Ferreira[1,2], Jonathan C Trinidad[1], Sunčica Šašić[1], Andrea G Hohmann[1,2], Dan Tracey[1,2] [1]Biology, Indiana University, [2]Gill Institute for Neuroscience, Indiana University

The venom of velvet ants (Hymenoptera: Mutillidae) is notoriously painful. The intensity of a velvet ant sting has been described as “Explosive and long lasting, you sound insane as you scream. Hot oil from the deep fryer spilling over your entire hand.” Velvet ant stings are an effective deterrent against potential predators across vertebrate orders, including mammals, amphibians, reptiles, and birds. This leads to the hypothesis that velvet ant venom targets a conserved nociception mechanism, which we sought to uncover using *Drosophila melanogaster* as a model system. *Drosophila* larvae have peripheral sensory neurons that sense potentially damaging (noxious) stimuli such as high temperature, harsh mechanical touch, and noxious chemicals. We found that velvet ant venom strongly activated *Drosophila* nociceptors through heteromeric Pickpocket/Balboa (Ppk/Bba) ion channels. Furthermore, we found a single venom peptide (Do6a) that activated larval nociceptors at nanomolar concentrations through Ppk/Bba. *Drosophila* Ppk/Bba is homologous to mammalian Acid Sensing Ion Channels (ASICs). However, the Do6a peptide did not produce behavioral signs of nociception in mice, which was instead triggered by other venom peptides that are non-specific and less potent in *Drosophila* nociceptors. This suggests that Do6a is an insect-specific venom component that potently activates insect nociceptors. Consistent with this, we showed that the velvet ant’s defensive sting produced aversive behavior in a predatory praying mantis. Together, our results indicate that velvet ant venom evolved to target nociceptive systems of both vertebrates and invertebrates, but through different molecular mechanisms.

543T **Loosely coupled oscillators as a correlate of behavioral control circuits within the central complex of Drosophila** Saul Garnell[1], Mehmet K Turkcan[2] [1]Computer Science, Auckland University of Technology, [2]Civil Engineering & Engineering Mechanics, Columbia University

In this study, we propose a novel methodology to model and analyze a circuit mechanism within the Fan-shaped body (FB), a core neuropil within the Central Complex of Drosophila melanogaster. Given the critical role of the Fan-shaped body in regulating both sleep and arousal in fruit flies, we hypothesize that specific neuronal circuits within Drosophila's FB function as loosely coupled oscillators, providing a control mechanism for these opposing behaviors. To test our hypothesis in silico, we developed a multi-step framework to identify neurons of interest performing as loosely coupled oscillators in the Central Complex using statistical pathfinding analysis, graph analysis, and simulated signal analysis approaches. The pathfinding analysis was central in identifying potential neuronal pathways and connections relevant to the regulation of sleep and arousal. Our study leverages publicly available computational tools to identify, visualize, and simulate neuronal signals, advancing the understanding of neural circuit dynamics and their implications for behavior and cognitive processes in Drosophila. The final results employ Wilson-Cowan model analysis between two neuron types, one excitatory and one inhibitory, elucidating how stable points demonstrated by Wilson-Cowan analysis act as neuronal attractors driving behavior. These findings can be applied to Bayesian Brain theories such as the Free Energy Principle and suggest experimental approaches to interpret the functionality of pathways identified in the Drosophila brain connectome.

544T **The Effect of Social Experience on Gene Regulation, Neural Activity and Behavior in *Drosophila melanogaster*** Chengcheng Du[1], Jesus Sotelo Fonseca[1], Ashley Jia[1], Shayna Scott[1], Marta Rozados Barreiro[1], Sumie Okuwa[1], Yuta Mabuchi[2], Nilay Yapici[2], Corbin D Jones[3], Pelin Volkan[1] [1]Duke University, [2]Cornell University, [3]UNC-Chapel Hill

Social behaviors of animals are modulated by signals from the environment. The molecular and neural circuit-based mechanisms underlying experience-dependent behavioral regulation remains poorly understood. Emerging evidence from both mammals and insects indicates the intimate connection between behavioral modulation, neural transcription, and neuronal activities. The *Drosophila melanogaster* is an excellent model where links among stereotyped courtship behaviors, genes and circuits have been elucidated. Transcription factors Fruitless and Doublesex control innate and learned male courtship behaviors of Drosophila, respectively. At the neural circuit level, a single cluster of P1 command neurons in the brain, which is both *fruitless* (*fru*) and *doublesex* (*dsx*) positive, drive male courtship behaviors. However, how social experience regulates master genes controlling courtship at the level of transcription in different courtship circuits remains unclear. Single-pair courtship assays showed that socially isolated male flies displayed more vigorous courtship behaviors compared to group housed males. The increase in courtship vigor in isolated males was accompanied by an increase in the response of P1 command neurons in the central brain. Single cell RNAseq of cells in courtship circuits of male flies raised under different social contexts showed that the transcriptome profiling of individual cells performs differently in different clusters. Behavioral screening with RNAi knocking down of those differentially expressed genes in specific cell types revealed promising candidate genes responsible for social experience dependent changes of courtship vigor, where genes involved in circadian regulation stood out significantly. In addition, disrupting pheromone receptor function in the olfactory system altered the expression level of neuromodulatory in both peripheral and central nervous system. These results suggest that social context alters gene expression in the courtship circuits partially through the olfaction system, and ultimately modifies neuronal activity and courtship behaviors.

545T **Interrelated insulin-like peptide and diuretic neuron regulate sex-specific aggression in *Drosophila*** Siyuan Yang Psychiatry, McLean Hospital-Harvard Medical School

Aggression is an evolutionarily conserved behavior essential for survival, enabling animals to secure resources, defend territory, and compete for mates. Both excessive and insufficient aggression can disrupt survival, making its regulation critical. While mammalian models present challenges due to the complexity of neural circuits, the fruit fly *Drosophila melanogaster* offers a powerful system for studying aggression. Its stereotyped, quantifiable behaviors and conserved genetic and neuromodulatory mechanisms provide valuable insights into the neural and molecular basis of aggression.

The connection between internal sugar levels and aggression remains poorly understood, despite its importance in linking metabolic states to behavior. To address this, we screened fly lines from the Janelia FlyLight collection using the GAL4-UAS binary expression system and identified two key neuronal subsets involved in internal sugar regulation. One line targets neurons under the control of a promoter linked to an insulin-like peptide (ILP), which regulates nutrient selection and sugar accumulation. The other line targets neurons under the control of a promoter associated with a diuretic hormone (DH), which mediates responses to food nutrients and starvation. ILP reflects the fly's energy balance, influencing aggression by signaling nutrient abundance, while DH senses nutrient scarcity, shaping behavior in response to starvation. Together, these peptides act as critical modulators of internal sugar levels and provide a framework for linking metabolic states to aggression.

Using the warmth-sensitive cation channel dTRPA1, we activated these neuronal clusters and observed changes in aggression intensity in both sexes. This study highlights the critical interplay between metabolic signaling and aggression and explores the neural circuits through which ILP and DH regulate aggressive behavior. By uncovering these mechanisms, we provide a foundation for future research into the neural circuit dynamics underlying the relationship between energy balance and aggression, with the goal of extending this understanding across species.

546T **Sublethal effects of agrochemicals on insects at environmentally relevant concentrations** Lautaro Gandara, Justin Crocker EMBL

Insect populations are declining at an alarming rate worldwide, and exposure to agrochemicals has been identified as a key factor affecting insect biodiversity and ecosystem health. Despite the prevalence of these chemicals, few studies have systematically evaluated their effects on insect behavior, physiology, and survival at environmentally relevant concentrations. We developed a high-throughput screening platform to assess the effects of >1000 agrochemicals - including insecticides, herbicides, fungicides, and plant growth regulators - on the behavior and physiology of Drosophila melanogaster. Our results showed that 57% of this library - many of them non-insecticide agrochemicals - significantly altered larval behavior at sublethal concentrations. Sublethal exposure to some of these compounds resulted in the accumulation of reactive oxygen species in the larval brain, reduced food intake, developmental delays, and reduced egg-laying rates. Additional experiments showed how different environmental conditions modulate these effects. For example, we observed that some of these effects were enhanced at higher temperatures, suggesting that global warming scenarios may exacerbate the harmful effects of agrochemicals. We also found evidence of similar adverse effects in other insect species, such as mosquitoes and butterflies. These findings highlight the need for updated environmental risk assessment protocols for agrochemicals that focus on sublethal and combinatorial effects on sentinel species tested under varying environmental conditions.

547T **Altered Vesicular Acetylcholine Transporter Expression Changes Acetylcholine Exocytosis in *Drosophila*** Rohina A. Nemat, Angeline-Claudia Atheby Biological Sciences, Delaware State University

Normal acetylcholine (ACh) exocytosis is required for the regulation of critical organismal functions like locomotion and learning and memory. Consequently, the alteration of cholinergic neurotransmission, either through increases or decreases in ACh release, impairs cognitive performance and locomotion ability. Significant advances have been made in elucidating how ACh is regulated in the brain in neuromuscular junction and yet despite this progress, the processes that regulate cholinergic abundance at the synaptic cleft are not fully understood. We are interested in shedding light on how one aspect of that machinery works. To this end, we are using the vesicular acetylcholine transporter (VAChT), which mediates the packaging and transport of acetylcholine (ACh) for exocytotic release, as a tool to understanding the mechanism through which acetylcholine release is regulated. We use both an overexpression of VAChT (using a construct that we have reported on previously) and mutants in *Vacht* that cause varying decreases in the gene's expression, to increase or decrease (respectively) the amount of ACh released into the synaptic cleft. And we are measuring the effect of that altered state on synaptic activity using a biochemical approach. We have optimized an assay that allows us to reliably measure cholinergic pathway components ACh and choline from as little as five *Drosophila* heads. Using this assay, we report the finding that consistent with its role in mediating ACh release, VAChT overexpression lines have an elevated total head ACh levels. Moreover, we report a significant increase in choline, the byproduct of the extracellular breakdown of ACh. In VAChT overexpressing flies, to determine the effect of altered VAChT expression of ACh level in vivo, we conducted an immunohistochemistry assay to determine the spatial localization of ACh and Vacht in both *Vacht* mutants and overexpressed specimens, using a spinning disk confocal microscopy and we found significantly elevated ACh staining at or near the plasma membrane of cholinergic neurons, as well as strong co-localization with Bruchpilot, an active zone marker. We present results of a quantitative analysis of the expression of ACh co-localized with that active zone marker, and we use this as a proxy for the level of ACh at the synaptic release site. Our data aligns with the results of our neurochemistry assay, indicating an elevated level of ACh in the overexpressed line and reduced of the neurotransmitter in the *Vacht* mutants level of ACh in mutant line. Collectively, these findings offer important information regarding how increases or decrease in vesicular transport of ACh affects its exocytosis in *Drosophila.*

548T **The cellular and molecular bases of sensory driven quenching of thirst in fruit flies** Anindya Ganguly[1], Arumoy OCAS Chatterjee[2], Ramandeep Singh[3], Shivani Swaminathan[3,4], Anant Zhaveri[3,5], Craig Montell[2] [1]Neuroscience Research Institute, University of California, Santa Barbara, [2]Neuroscience Research Institute; Molecular, Cellular and Developmental Biology, University of California, Santa Barbara, [3]University of California, Santa Barbara, [4]Foothill High School, [5]Brown University

Dehydration triggers the sensation of thirst, a response aimed at reversing water loss and preventing health consequences. While the initiation of thirst is driven by internal physiological states, the quenching of thirst results from both the initial detection of water by the sensory system and by the consequent changes in an animal's internal state following absorption of the water. While it is well known that it is critical to consume water to prevent dehydration, it is also essential to properly quench thirst to maintain fluid homeostasis in the body. Severe deficit in thirst quenching (polydipsia) is characterized by excessive water consumption, causing polyuria, hyponatremia, and in the most extreme cases, water intoxication, which can be fatal. Despite the importance of thirst-quenching, we have only a rudimentary understanding of the cellular and molecular basis through which peripheral sensory neurons contribute to thirst-quenching. Here we report that *OtopLA*, a *Drosophila* homolog of the Otopetrin family of proton channels underlie sensory-driven water satiety or thirst quenching. To investigate the roles of the gustatory system and OtopLA in pre-absorptive thirst quenching, we compared the drinking behavior of wild-type and *OtopLA* mutant flies, when presented with water. Remarkably, *OtopLA* mutants exhibited a significantly prolonged drinking duration relative to wild-type, indicating a defect in water satiety. Restoring *OtopLA* expression in one class of gustatory receptor neurons (D or high salt sensing GRNs) re-established normal drinking duration in the mutants. We observed that the frequency of water-induced action potentials was reduced in the D GRNs of *OtopLA* mutants, suggesting that *OtopLA* function in these neurons is necessary to signal water satiety. Expressing a water receptor in the D GRNs of *OtopLA* mutants restored normal drinking duration suggesting that *OtopLA* could also be acting as a water sensor. Notably, expressing mouse *Otop1* in *OtopLA* neurons restores normal drinking duration in *OtopLA* mutants, suggesting that *mOtop1* may also serve as a water sensor and regulate water satiety. We further identified a role of *ppk23*, a DEG-ENaC channel expressed in the D GRNs in water satiety. However, whether *ppk23* functions independently or in conjunction with OtopLA is subject to future investigation. This study provides new insights into the cellular and molecular mechanisms underlying water satiety, with potential implications for addressing disorders of excessive water intake. Additionally, it discovers a novel, potentially conserved role of Otopetrins as water sensors.

549T **Molecular Basis of Flavonol Sensing in Fruit Flies** Zixin Lu[1], Anindya Ganguly[2], Ramandeep Singh[1], Craig Montell[3] [1]University of California, Santa Barbara, [2]Neuroscience Research Institute, University of California, Santa Barbara, [3]Neuroscience Research Institute; Molecular, Cellular and Developmental Biology, University of California, Santa Barbara

Fruit flies rely on their sense of taste to locate food and avoid ingesting toxic substances. Flavonols, a class of natural compounds abundant in fruits and vegetables, represent a key chemical cue in the natural environment of fruit flies. Despite their ecological relevance, the molecular and cellular mechanisms by which the peripheral taste system detects flavonols remain unclear.

To investigate fruit fly gustatory responses to flavonols, we focused on catechin, a widely occurring flavonol found in natural sources such as green tea leaves, cacao, and grapes. In feeding behavior assays catechin induced gustatory aversion, and suppressed sugar-induced proboscis extension responses in wild-type flies.

Previous work from our lab has demonstrated that rhodopsins, typically associated with photoreception, function as taste receptors for certain naturally occurring bitter compounds in flies. To explore whether rhodopsins are involved in catechin detection, we screened all *rh1–7* mutants in behavioral assays. Our results identified *rh5* as essential for both gustatory aversion and peripheral sensitivity to catechin.

Since the ion channel *TRPA1* has been shown to act downstream of rhodopsins in gustatory perception, we examined its role in catechin detection. *TRPA1* mutants exhibited a complete loss of both aversive behavior and peripheral sensitivity to catechin, highlighting its critical role in this pathway. To identify the specific isoform required, we rescued various *TRPA1* isoforms in the mutants and observed that expressing *TRPA1-D* restored behavioral aversion. Furthermore, two-electrode voltage-clamp recordings in *Xenopus laevis* oocytes expressing *TRPA1-D d*emonstrated direct activation by catechin, supporting a model in which *TRPA1* detects catechin both directly and downstream of rhodopsins. However, the precise sensitivity of *TRPA1* and *rh5* to catechin requires further investigation.

TRPA1 is highly conserved across phylogeny, suggesting that catechin may elicit aversive responses in other *Drosophila* species. To test this, we studied *Drosophila suzukii*, an invasive pest that lays eggs on ripening fruits. Oviposition assays revealed that coating blueberries with catechin significantly reduced egg-laying by *D. suzukii*. These findings suggest the potential of using certain flavonols as natural, eco-friendly alternatives to harmful insecticides in agriculture.

550T **Serotonergic receptors drive graded presynaptic plasticity of acetylcholine release in Kenyon cells following associative learning** Aaron Stahl[1], Valentina Botero[1], Seth Tomchik[1,2,3] [1]Neuroscience and Pharmacology, University of Iowa, [2]Pediatrics, University of Iowa, [3]Iowa Neuroscience Institute, University of Iowa

Precise temporal control of multiple monoaminergic neurotransmitters underlies associative learning. *Drosophila* olfactory associative learning drives changes in neurotransmission of presynaptic acetylcholine (ACh) release from mushroom body Kenyon cells (KCs). These changes in ACh release are valence specific across the longitudinal axonal compartments of the KCs. The compartments are innervated by unique downstream mushroom body output neurons (MBONs). In addition, each compartment receives heterosynaptic input from different sets/combinations of monoaminergic neurons. These monoaminergic neurons modulate the synaptic neurotransmission from the presynaptic KCs to the downstream MBONs. Along with dopamine, serotonergic neurons innervate the KC compartments, and KCs express multiple serotonin receptors. Serotonergic neurons exert strong behavioral effects on olfactory classical conditioning, though their role in modulating the compartmentalized synaptic plasticity driven by reward learning is not known.

Here we investigate the role of serotonin (5-HT) in driving reward-associated presynaptic plasticity in KC compartments. We paired *in vivo* imaging of ACh release across the MB γ lobe with appetitive conditioning and conditional RNAi-mediated knockdown of 5-HT receptors in the KCs. Conditional knockdown of two different 5-HT receptors in adult mushroom body Kenyon cells altered the odor-evoked release of ACh across γ lobe compartments following appetitive conditioning, systematically shifting baseline odor-evoked ACh release and abolishing the conditioning-induced changes in ACh release across compartments. This loss of plasticity correlated with loss of behavioral learning – appetitive conditioning was impaired following the knockdown of different 5-HT receptor subtypes. These data suggest that layered signaling via monoaminergic pathways modulates baseline ACh release and drives plasticity underlying learning.

551T **Investigating the Interactions between Metals and Amyloid Precursor Protein-Like (APPL) on Aging-Dependent Locomotor Decline in *Drosophila melanogaster*** Justine Anne A Guevarra, Fernando J Vonhoff Biological Sciences, University of Maryland Baltimore County

Amyloid precursor protein (APP) is a single-pass transmembrane protein encoded by the human *APP* gene. It is expressed in several tissues and organs, but it is primarily found in the brain. Its physiological functions remain elusive, but APP is thought to be involved in various biological processes including neuronal development and survival, learning and memory, synaptic plasticity and transmission. Studies on APP reveal its potential protective role in neurodegeneration. An example of neurodegenerative disorder associated with APP is Alzheimer's Disease. APP is highly conserved across species with related proteins such as APLP1 and APLP2 in mammals, and APPL in fruit flies. Studies in mice and flies show that lack of APP proteins results in developmental and locomotor deficits, which can be rescued by the expression of the human APP, confirming its conserved properties. APP is also a metalloprotein and has metal-binding domains for copper (Cu) and zinc (Zn) ions. Moreover, APP mRNA contains iron-response elements (IRE) in its structure that can bind to iron-regulatory proteins (IRP), allowing intracellular iron (Fe) to regulate APP translation. Interestingly, Fe shares similar chemical characteristics with aluminum (Al), allowing Al to bind to iron-binding proteins involved in transporting Fe, which can disrupt Fe homoeostasis. Additionally, recent studies display increasing evidence on the possible link between metals and neurodegenerative disorders. This project aims to investigate the interactions between metals and APPL in aging-dependent locomotor decline in the model organism, *Drosophila melanogaster*. Overall, the knowledge gained will serve as a reference for future studies in mammals and broaden the understanding of the fundamental mechanisms involving APP, metals, and aging in flies.

552T **Multimodal integration of skylight cues for navigation in the Drosophila central complex** Sharon J. Su[1], Emil Kind[2], Mathias Wernet[2], Larry F Abbott[3], Rudy Behnia[3] [1]Neurobiology and Behavior, Columbia University, [2]Free University of Berlin, [3]Columbia University

Many aspects of sensory information are linked in the natural environment, and evolution has shaped different navigational strategies across species to take advantage of this. A well-known example is skylight navigation in insects. For instance, fruit flies use the position of the sun to navigate, but they can also orient successfully under the daytime sky even when the sun is not visible. This robustness is possible due to other global skylight cues—such as polarized light, chromatic contrast, and intensity gradients—which all change in a stereotyped manner with respect to the position of the sun. Recent studies in Drosophila have shown that visual information about angles of polarized light (AOP) is conveyed to the central complex, the navigational center of the brain, consistent with previous behavioral work showing that flies orient with respect to changing AOP. However, while the AOP pattern in the sky can be used to infer a path that the observer and the sun lie on, it cannot specify which direction along that path the sun is located due to its symmetry. Thus, flies must integrate another asymmetrical skylight cue to resolve this directional ambiguity. To investigate this, we built a biologically constrained, connectomics-based circuit model of the sky compass pathway to observe how the system would behave under the natural sky. Our findings indicate that combining chromatic contrast and polarization allows for successful, robust navigation. Using in vivo calcium imaging, we show chromatic tuning in ER4m, a visual ring neuron located in the central complex that has been shown to provide skylight polarization information into the E-PG compass system. Specifically, ER4m is color opponent, i.e. inhibited by long wavelengths of light and excited by short wavelengths. Furthermore, ER4m also responds linearly to ethologically relevant chromatic contrast changes, whereas its response to intensity changes is sigmoidal and approaches a ceiling at low relative intensity changes. The encoding of chromatic and intensity information in ER4m demonstrates that these cues are integrated before E-PG compass neurons, suggesting these secondary skylight cues are used to resolve the AOP pattern's directional ambiguity in the Drosophila sky compass.

553T **The Neural Basis of Directional Escape in Drosophila Larvae** Abby J Wood[1], Leeza Pesok[2], Wesley B Grueber[1] [1]Neuroscience, Columbia University, [2]Barnard College

Animals exhibit innate escape behaviors that allow them to evade predators and other noxious cues in the environment. The sensory neurons that evoke escape, called nociceptors, are well studied, as well as some interneurons in the nervous system that are involved in escape; however, how escape motor programs are flexibly coordinated at the circuit level is not fully understood. In response to a parasitic wasp attack or other noxious stimuli, *Drosophila* larvae perform sequential bending and rolling involving one of four different motor patterns. Larvae under attack exhibit directional escape responses depending on where the stimulus originates, which may confer an evolutionary advantage for efficiently fighting off the wasp. How specific escape directionality is determined is not well understood. To examine evoked escape behavior, we used the SPARC system to sparsely activate one or both bilateral escape command neurons (Goro) with optogenetics. Consistent with prior studies, we find that activation of both Goro neurons via SPARC leads to consistent activation of escape behavior, but no specific directional preference. Unilateral excitation significantly reduced directional changes during escape that can occur during bilateral Goro activation. We find that unilateral activation biases roll direction, but the laterality of Goro activation does not dictate bend direction, uncoupling these two aspects of escape behavior. Further, unilateral activation of Goro results in a lower probability of escape and delayed initiation of rolling compared to bilateral activation. Together, our data suggest that Goro command neurons may flexibly modulate escape action selection in response to directionally specific sensory stimuli. To this point, we have sparsely activated primary nociceptors to ask whether laterality of their activation results in either bend or roll direction preference. Preliminary analysis of the larval CNS connectome has revealed a potential premotor circuit for directional roll initiation during larval escape. Thus, sparse or single neuron activation within a cell type can bias behavioral outputs, and this approach may aid in understanding the roles of specific neurons in other somatosensory circuits. Future experiments will ask if specific nociceptive interneurons encode directional bending during escape to gain a complete understanding of how flexible escape motor programs are coordinated at the circuit and behavioral level.

554T **The effect of social isolation on the dopamine system and behavior through pheromone perception** Lanling Jia, Sumie Okuwa, Pelin Volkan Duke University

Social isolation induces various behavioral and physiological changes across species, suggesting a potentially conserved mechanism underlying changes in motivational state that adapts to changes in sensory information associated with social contact. Because dopamine is known as the key molecule that regulates motivation and thus has a pleiotropic effect in various behaviors, this study seeks to use Drosophila melanogaster as a model to elucidate the neural mechanisms underlying social isolation-induced behavior changes, focusing on olfactory-driven dopamine circuit modulation. I hypothesize that the pheromone circuits detect social cues and modulate dopaminergic neuron activity to change behavior motivation. To test this hypothesis, I will perform in vivo assays to examine the changes in activity within olfactory sensory circuits and dopaminergic neurons with social experience and measure behavioral responses. I will perform in silico assays using connectome data to map the anatomical and functional connectivity within and between the olfactory system and dopaminergic neurons. I will functionally and genetically manipulate neural activity and test its effects on behavioral responses to social experience. This research will provide critical insights into the olfactory-driven modulation of dopamine circuits, contributing to a deeper understanding of the neural encoding that mediates behavioral consequences of social experience and isolation.

555T **Understanding Temperature Compensation Using a Transcriptomics Approach** Wanhe Li[1], Fumihiro Ito[2] [1]Biology, Texas A&M University, [2]Texas A&M University

Temperature, due to its impact on various biological processes, induces organisms to adapt through temperature-dependent alterations in behavior and physiology. Conversely, the circadian rhythm maintains its free-running periodicity across a broad temperature range, known as temperature compensation. In recent years, scientists have been uncovering the temperature-dependent modulation of the molecular clock machinery underlying temperature compensation. However, temperature affects not only the molecular clock but also various other aspects of biological processes, such as metabolism and stress responses. Therefore, it remains unclear whether temperature compensation applies to these processes regulated by the circadian rhythm.

This study used the temperature-sensitive ectotherm, *Drosophila melanogaster*, to further understand temperature compensation. We sampled flies kept under conditions of constant darkness and in conditions of darkness with either low or high temperatures to explore the periodicity of gene expression on the transcriptome level. Performing time-series RNA sequencing from these samples, we analyzed differentially expressed genes sensitive or insensitive to low or high temperatures, as well as groups of genes exhibiting differential oscillating properties. Our analyses provide insights into the categorization of an extensive diversity of biological processes that are temperature-compensated or temperature-sensitive.

556F **Ionic homeostasis in Astrocyte-like glia regulates olfactory perception** Shreya Mandal, Abhijit Das Bioscience and Biotechnology, Indian Institute of Technology Kharagpur

An organism relies on its senses of hearing, taste, touch, smell, and vision to translate external environmental conditions into internal representations. Neurons are widely regarded as the primary regulators of sensory function, but the brain also contains another critical component: the glial cells. Astrocyte glia, in particular, send branches that are closely associated with synapses and have been shown to modulate neuronal information processing.

In this work, we investigate the modulatory role of astroglial cells in orchestrating the ionic homeostasis of neurons and their contribution in sensory perception. Astrocytic calcium levels were manipulated in two ways: by elevating calcium through the expression of the *dTRPA1* transgene and by reducing it via RNAi-mediated knockdown of the voltage-gated calcium channel subunits *Caα-1D* and *Cacophony*, driven by *Alrm*-GAL4 in all astrocyte-like glial cells. We observe a significant impairment of the odor perception behavior of the flies when tested both for aversive and attractive odorant. These results provide evidence of the role of astrocytic calcium homeostasis in neuromodulation.

In our experiment with potassium ion regulation, we expressed the potassium channel *Kir2.1* and this approach led to a significant olfactory perception defect, further implicating importance of potassium homeostasis in astrocyte function. In contrast, chloride ion modulation via the optogenetic chloride channel *GtACR1* did not impair olfactory detection fidelity, suggesting that astrocytic calcium and potassium homeostasis are specifically crucial for modulating neuronal activity and maintaining sensory processing.

Next, we investigated the influence of astrocytic calcium on the balance of neuronal excitation and inhibition. Interestingly, we found that knockdown of the GABA uptake channel GAT in astrocytes led to a pronounced olfactory perception defect. We are currently exploring the role of endocytic pathways in regulating GAT levels on astrocyte membranes and their impact on olfactory perception. Future work will involve transcriptomic analysis of astrocytes to elucidate the full repertoire of ion channels involved in ionic homeostasis.

557F **Chemosensation inhibits cannibalistic behavior in *Drosophila* larvae** Nagisa Matsuda[1], Masato Tsutsumi[2], Misako Okumura[1], Takahiro Chihara[1] [1]Graduate School of Integrated Sciences for Life, Hiroshima university, [2]Graduate School of Medicine, Nagoya university

Feeding behavior is essential for the survival of animals. Animals exposed to nutritional stress, such as starvation, may prey on other animals of the same species to compensate for nutritional deficiencies. This predatory behavior, particularly cannibalism, is considered to be an innate and rational behavior. Cannibalism has been observed in a wide range of animal groups, from invertebrates to vertebrates including *Drosophila melanogaster* larvae. *Drosophila* larvae do not show predatory behavior under normal conditions, but exhibit cannibalistic behavior at very high population densities or when exposed to starvation. Because cannibalism involves feeding on conspecifics, it is thought that there are cognitive modalities associated with cannibalism, such as mechanisms that recognize other individuals as conspecifics or mechanisms that promote or inhibit cannibalism in a group. However, the molecular control mechanisms of cannibalistic behavior remain largely unknown.

In this study, we found that a double mutant of olfactory and gustatory sensory receptors in *Drosophila* larvae exhibits cannibalistic behavior. The double mutant larvae showed no developmental delay when reared individually, but showed a significant developmental delay when reared in groups. Furthermore, when the control strain (*white*) and the double mutant larvae were reared on the same medium, the developmental time of the control strain was significantly delayed. These results suggest that the larvae of the double mutant larvae are responsible for the developmental delay of the other individuals. Furthermore, detailed observation of larval behavior revealed that the double mutant exhibited cannibalistic behavior. In the double mutant, the number of mouth hook contractions over the body surface of other larvae was significantly increased compared to controls. This result suggests that the double mutant is motivated to feed on other larvae. A quantitative analysis of inter-individual interactions showed that the double mutant has altered responses to other individuals. We hypothesize that the cannibalistic behavior of the double mutant causes stress and damage to other individuals, resulting in the developmental delay. In this presentation, we will discuss how chemosensory stimulation suppresses cannibalistic behavior in *Drosophila* larvae.

558F **Association of a single odor with toxic/bitter food elicits an olfactory aversive conditioning memory in *Drosophila*** Snehasis Majumder[1], Biswajit Chakraborty[2], Gaurav Das[3], Abhijit Das[1] [1]Bioscience and Biotechnology, Indian Institute of Technology Kharagpur, [2]Heinrich Heine University Düsseldorf Germany, [3]National Centre for Cell Science (NCCS), Pune

Encoding of experiences in the form of memory and their future retrieval is fundamental for the survival of any organism. The brain is an ensemble of neuronal circuits performing complex cognitive processes. A neuronal circuit confers the ability to learn from experiences, leading to improved performance or potential for survival in a dynamic environment by shaping behavioral plasticity. Associative memory is a complex learning outcome where the brain correlates between two unrelated events. With repeated or prolonged training, memories can last for long, sometimes for a lifetime. Long-term memory is associated with synaptic plasticity through transcriptional activation of memory genes, which is linked with chromatin remodeling.

A “single odor – toxic food” gustatory aversive conditioning memory paradigm has been standardized in this study where an attractive odorant has been coupled with $CuSO_4$, a bitter-tasting toxic substance. A spaced training paradigm triggers the formation of long-term associative memory in the adult fly as well as in larvae, eventually reducing their attraction towards the odorant, which is quantifiable in a simple binary odor choice assay. I observe that rigorous training with an increasing number of training cycles, triggers stronger memory. This memory, induced in adults, is dependent on *Creb-regulated* transcription, new protein synthesis and shows a gradual decay pattern over the course of 7-8 days, confirming the elicited memory to be ‘long-term memory (LTM)’. Conversely, similar training cycles in larvae, which harbor comparatively simpler odor processing circuit, trigger a vigorous repulsion towards the conditioned attractive odorant. In classical associative conditioning, usually two odors are used to train and assess the memory score for learning; restricting the probability of observing the odor valence inversion after training, which is possible with this single-odor paradigm. Temporal blocking of the synaptic transmission in specific neuronal subsets demonstrates that, the formation as well as the consolidation of this avoidance memory depends on the mushroom body neurons and their negative reinforcement mediated by the dopaminergic input (PPL1 neurons). This memory paradigm is also used to observe memory impairment in major tauopathies. I am exploring the dynamics of chromatin remodeling in the mushroom body neurons during this LTM formation.

559F **Modeling chemical induced tissue damage and nociception in *Drosophila* larva through repeated acid exposure** Jaime A Arroyo, Raul Chavez, Vanessa Pando, Jacob Jaszczak Biology, New Mexico State University

Drosophila larva rely on their nociceptive sensory system to detect and respond to potentially damaging stimuli. Numerous forms of damage to the larval epidermis can lead to hypersensitivity through allodynia or hyperalgesia (DOI: 10.1002/dvdy.22737). High concentrations of hydrochloric acid can induce nociceptive behavior in larvae. Additionally, physical injuries that breach the larval cuticle, such as puncture wounds, induce allodynia by enhancing larval sensitivity to normally sub-threshold acid concentrations. In contrast, non-cuticle breaching injuries, such as UV irradiation or pinch wounds, fail to elicit allodynia, suggesting that the larval cuticle may need to be broken to cause acid induced allodynia. However, acid exposure itself produces damage to the larval epidermis and sensory neurons (DOI: 10.1098/rstb.2019.0282). Therefore, we investigated the effects of chemical-induced tissue damage on nociception in larvae. We find that while acid exposure does not induce allodynia or hyperalgesia, repeated acid exposure produces nociceptive hypoalgesia. Multidendric (md) neurons in the larva body wall have previously been shown to be necessary for chemical nociception (DOI: 10.1098/rstb.2019.0282), and we find that acutely blocking md neuron synaptic transmission also reduces acid nociception. Using a PBac insertion allele of *Painless*, we find that this mutation increases the severity of hypoalgesia after repeated acid exposure, suggesting that two parallel mechanisms for sensing acid nociception may be present in larva. Ongoing experiments are seeking to determine the pathway damaged by acid exposure, as well as the roles of *TrpA1* channels and *painless* in maintaining nociceptive sensitivity after chemical injury. This study aims to deepen our understanding of the genetic and cellular mechanisms that drive adaptive responses which maintain nociception after tissue injury.

560F **Age-related dysregulation of cAMP signaling in *Drosophila* taste circuits** Rose Riley, Mary Gekoskie, Yvette Obediente, Elizabeth Brown Florida State University

Deficits in chemosensory processing are associated with healthy aging and are often the first to decline in age-related and neurodegenerative diseases. Understanding the impact of age on chemosensory circuit plasticity will provide valuable insights into the dysregulation of these processes over time. Despite our extensive knowledge of the taste system in both mammals and invertebrates, the processes underlying a decline in taste processing with age remain largely unexplored. To determine whether the effects of aging on taste are conserved in flies, we compared the response of flies to different appetitive tastants. Aging impaired response to sugars, but not medium-chain fatty acids that are sensed by a shared population of neurons, revealing modality-specific deficits in taste. Selective expression of the human amyloid beta (Ab) 1-42 peptide bearing the Arctic mutation (E693E) associated with early onset AD in the neurons that sense sugars and fatty acids phenocopies the effects of aging, suggesting that the age-related decline in response is localized to gustatory neurons. Sugars and fatty acids activate overlapping populations of GRNs but utilize distinct second messenger signaling pathways, suggesting that age-related taste deficits may stem from dysregulation in second messenger signaling pathways in sweet taste neurons. We are testing this hypothesis by performing functional imaging of cAMP activity in sweet-taste neurons. To determine whether an increase in cAMP activity can prevent or restore age-dependent taste decline, we are pharmacologically and transgenecially manipulating cAMP activity in sweet taste neurons and are measuring sweet taste response in aged flies. Our results shed light on the molecular mechanisms that regulate age-dependent declines in taste. By establishing the *Drosophila* taste system as a model for investigating age-dependent neural dysfunction, these findings can be translated to models of neurodegenerative and age-related diseases that also involve loss of chemosensory function.

561F **Mechanisms of Capsaicin Tolerance and Sensory Adaptation in TRPV1-Lacking Drosophila melanogaster** Gerardo Flores-Iga[1], Mohankumar Amirthalingam[1], Preeti Kayastha[1], Carlos Lopez-Ortiz[1], Purushothaman Natarajan[1], Padma Nimmakayala[1], Elizabeth Brown[2], Umesh Reddy[1] [1]Department of Biology, West Virginia State University, [2]Department of Biological Sciences, Florida State University

Unlike mammals, invertebrates such as *Drosophila melanogaster*, which lack the Transient Receptor Potential V member 1 (TRPV1), respond to capsaicin through alternative chemosensory mechanisms, and are not naturally averse to spicy compounds. This study finds that wild-type flies are attracted to capsaicin-containing food, while the TRPV1 expression in multidendritic neurons induces complete aversion. Capsaicin disrupts sleep in TRPV1-expressing flies but promotes deep sleep patterns in wild-type flies, particularly males during nighttime. To explore capsaicin sex-specific effects, we performed deep brain transcriptomics on wild-type and TRPV1- humanized flies on capsaicin diets. Results revealed upregulation of odorant-binding proteins and receptors in wild-type flies, suggesting a capsaicin-associated detection reflex. Genes involved in metabolism, defense, reproduction, circadian rhythm, and longevity (e.g., *fit*, *Dgat2*, *Ctsk*, *Att-s*, *Jhbp12*, *timeless*, *period*, and *CYP450*s) were also differentially expressed, reflecting adaptations in response to capsaicin. At the single-nuclei RNA-seq level, *Obp56e,* a possible capsaicin carrier to chemosensory receptor neurons, and *CG8343*, a carbohydrate binding protein, were upregulated. We also observed differential expression of *insulin-like peptides 2* and *3*, linking capsaicin to glucose metabolism and sleep regulation. These findings highlight capsaicin tolerance and chemosensory mechanisms in TRPV1-lacking invertebrates, broadening our understanding of chemosensation beyond vertebrate models and revealing evolutionary adaptations in taste, metabolism, defense, and circadian pathways in response to environmental chemicals.

562F **Understanding the behavioral and transcriptional role of KDM5 in the *Drosophila* mushroom body** Amira Mahoney[1,2], Bethany Terry[1], Hayden Hatch[2,3], Julie Secombe[1,2] [1]Genetics, Albert Einstein College of Medicine, [2]Neuroscience, Albert Einstein College of Medicine, [3]Child Neurology, Boston Children's Hospital

KDM5C is a lysine demethylase essential in transcriptional regulation. While KDM5C is ubiquitously expressed, its function is particularly important in neurons, as pathogenic variants in this gene cause KDM5C-Associated X-linked Intellectual Disability (KDM5C-XLID). KDM5C-XLID is characterized by gross developmental delay, intellectual disability, epilepsy, and sleep disturbances. Why loss of KDM5C-mediated gene regulation leads to altered neurological development and function remains unknown. Approximately 80 unique variants have been described in KDM5C-XLID, however clinical understanding has become complicated for patients who have a "variant of uncertain significance". Revealing these variants and potential links to reported phenotypes can enrich our understanding of these genetic differences and offer comprehensive diagnostic and therapeutic strategies for patients. Additionally, significant learning and memory disparities occur in the setting of loss of protein function. Using Drosophila as a genetic model of KDM5C-XLID, our lab has demonstrated that loss of KDM5 results in significant functional compromises to translation efficiency and long- term memory. Memory and learning are mediated by the fly mushroom body, a brain region analogous to the mammalian limbic system. Specifically, long-term memory is traditionally mediated by mushroom body alpha and beta neurons, while short-term memory is mediated by gamma neurons. While the functional and transcriptional role of KDM5 has been explored in alpha and beta neurons, little is known on how loss of KDM5 in gamma neurons affects morphological, functional, and molecular outputs. I will study KDM5C-XLID through exploring molecular programs involved in short term memory and learning defects in the gamma neurons of Drosophila. I hope to reveal additional genetic variants can be recapitulated in the Drosophila model to understand phenotypic patterns, and elucidate the underlying molecular programming affected by loss of KDM5. Ultimately, this path will streamline diagnostic pipelines, reveal therapeutic strategies, and strengthen our understanding of the molecular pathways involved in KDM5C-XLID.

563F **Complete knockout of serotonin transporter (*sert*) in *Drosophila melanogaster* increases baseline sleep, fragments deep sleep length, and enhances starvation resistance.** Marciella V Shallomita[1], Elaine Miranda Perez[1], Abigail L Forrest[1], Sadie M Oesch[1], B. Jill Venton[2], Jeffrey M Copeland[1] [1]Biology, Eastern Mennonite University, [2]Chemistry, University of Virginia

The monoamine serotonin is known to impact human behaviors such as sleep cycles, eating, anxiety, and depression. The reabsorption of serotonin back into the presynaptic neuron is mediated by the serotonin transporter (SERT), the target of selective serotonin reuptake inhibitors (SSRIs) used to treat depression. To understand the full activity of the transporter, we created a sert knockout allele (*sert*$^{\Delta 3.9}$) in *Drosophila melanogaster* that removes 3.88 kb of the gene's coding region. A multiple sequence alignment between *Drosophila melanogaster*, *Danio rerio*, *Homo sapiens*, *Mus musculus*, and *Gallus gallus* shows that the deletion covers the most conserved protein sequence across these species. The *sert* mRNA and protein are undetectable by qPCR and Western blot. Compared to the control Canton-S flies, *sert*$^{\Delta 3.9}$ mutant flies exhibit a 128.7% significant increase in sleep during both light and dark phases of a 12:12 LD cycle. Interestingly, long sleep (>25 minutes) is more fragmented by 28% compared to control flies. The *sert*$^{\Delta 3.9}$ flies are significantly less active flies, with a 91.0% decrease in activity compared to the control. These flies also show increased starvation resistance, together with a decrease in feeding but no change in mass. The observed decrease in feeding with no change in weight suggests an adaptation that allows the flies to withstand periods of food scarcity better. The *sert*$^{\Delta 3.9}$ allele represents a true knockout of the *sert* gene and can be used to fully understand the gene's function in sleep, feeding behavior, and starvation.

564F **Wingless pathway affects nociceptive sensitivity** Michael Caterina[1], Finn Sclafani[1,2], Luke M Jenkins[2], Trevor J Flanagan[2], Zachary A Ahmida[2], Camilla AR Lattanzi[2], Dawson A Turcotte[2], Connor E Nowak[2], William C Harriman[3], Christine M Hale[4], Lindsey A Fitzsimons[5], Julie K Moulton[1], Kerry L Tucker[5], Geoffrey Ganter[2] [1]College of Arts and Sciences, University of New England, [2]University of New England, [3]University of Maine at Farmington, [4]Graduate School of Biomedical Science and Engineering, University of Maine, [5]College of Osteopathic Medicine, University of New England

Chronic pain affects an estimated 1.4 billion adults worldwide, with more affected by other forms of abnormal pain. Currently opioids are the best treatment for abnormal pain, but come with the risk of addiction and other side effects. Using *Drosophila melanogaster* as a model, alternative drug targets for reducing abnormal nociceptive hypersensitivity could be found in the known metabolic pathways of the animal model and transferred to humans in potential treatment strategies.

Previous research has identified many components in the Wingless developmental pathway and its mammalian counterpart Wnt. We hypothesized that the pathway also plays a role in affecting nociceptive sensitivity in the larval *Drosophila* model. To test this hypothesis, candidate components of the Wingless pathway were cell-specifically underexpressed or overexpressed. This was accomplished using Gal4/UAS and RNA interference tools to manipulate gene expression in the Pickpocket-expressing Class 4 multidendritic neurons, considered to be primary nociceptors. Behavioral thermal and mechanical nociception assays were conducted on 3rd instar larvae, probing the function of the transmembrane receptor Arrow, the transduction proteins Gilgamesh and Disheveled, the downstream transcriptional target Senseless, and the intraflagellar transport protein NompB, required for primary cilium assembly.

Results showed nociceptive hypersensitivity resulting from overexpression of Gilgamesh, and hyposensitivity resulting from underexpression of Arrow, Gilgamesh, and Senseless. Nociception assays also revealed hyposensitivity resulting from underexpression of NompB, suggesting for the first time that a primary cilium is present on the nociceptors of Drosophila and that this organelle plays a role in establishing nociceptive sensitivity. Underexpression of Disheveled, thought to connect the primary cilium with the Wingless pathway, also led to behavioral hyposensitivity. The effects of these Wingless/Wnt pathway manipulations on dendritic arborization morphology was also studied. Taken together, these results support the hypothesis that the Wingless pathway promotes nociceptive sensitivity in the *Drosophila* larva. This work suggests that the human homologs of the pathway and its associated components represent promising potential drug targets.

565F **Investigating integration of visual information for navigation in the *Drosophila melanogaster* sky compass pathway** Sarita Padukone[1], Sharon Su[1], Rudy Behnia[2] [1]Neuroscience, Columbia University, [2]Columbia University

Spatial orientation is a fundamental cognitive function crucial for survival. Multiple environmental cues contribute to this process, providing information for determining direction. Studies of insects that rely heavily on navigation, such as desert ants, locusts, monarch butterflies, and dung beetles, demonstrate that they orient themselves using various skylight cues, including the sun's position, sky polarization patterns, and gradients of light intensity and color in the sky. Although it is well established that these visual cues are used in combination, we still lack a critical understanding of how they are encoded and integrated within neural circuits. In this work, we use *Drosophila melanogaster* to explore multimodal sensory integration during navigation. The recently mapped fly connectome has revealed a "sky compass" pathway, a specific neural circuit that relays visual information to the ellipsoid body, which serves as the fly's navigation center. Sky light detection begins in the upper portion of the fly's compound eye, known as the dorsal third (D3), where ommatidia face upwards. The dorsal rim area (DRA), which includes the uppermost row of ommatidia in each eye, is particularly sensitive to polarized light. Previous studies show that through this sky compass pathway, photoreceptors in the DRA transmit polarized light information to the ellipsoid body, playing a key role in navigation. While the role of polarization detection in this pathway has been well studied in flies, other visual modalities remain largely unexplored. Our research aims to investigate how additional skylight information from photoreceptors across the broader D3 region contributes to the sky compass pathway. We hypothesize that, like other insects, flies use not only polarized light but also color and light intensity cues for navigation, with these signals encoded within the sky compass pathway. Our aim is to understand how these additional cues are encoded and integrated in the sky compass pathway and how they inform the fly's internal compass. Using two-photon calcium imaging and a custom built color mixer, we investigate how visual cues converge to guide navigation in *Drosophila melanogaster*, offering insights into sensory integration across species.

566F **Variation in Mushroom Body Morphology in Cocaine Preferring Drosophila Genetic Reference Panel Lines** Alp Mete Ummet, Trudy Mackay, Robert Anholt Genetics & Biochemistry, Clemson University

Previous studies revealed natural genetic variation in cocaine consumption and preference among the wild-derived, inbred lines of the Drosophila Genetic Reference Panel and implicated the mushroom bodies, brain structures that mediate experience-dependent behavior (Highfill et al., PLoS Genet. (2019) 15, e1007834). Previous studies also showed correlations between variation in mushroom body structure and aggression (Zwarts et al., Nat. Commun. (2015) 6, 10115). To assess whether variation in mushroom body morphology is correlated with variation in cocaine preference, we selected DGRP lines in which at least one sex showed preference for a cocaine-supplemented sucrose solution and control lines with mean aversion scores for cocaine preference. We dissected brains and stained mushroom bodies from males and females separately with an anti-fasciclin-II antibody. We quantified morphological measurements of lengths and thicknesses of the alpha and beta lobes by three-dimensional confocal microscopy. We also observed the absence of lobes, bilateral asymmetry, and anatomical abnormalities. Our initial studies on a small set of six lines, three cocaine-preferring lines and three lines with mean aversion scores, showed variation in mushroom body morphology and suggested a correlation of cocaine preference with alpha lobe structure. To consolidate these observations with statistical significance, we will expand this initial study to a larger sample of 48 lines. Based on evolutionary conservation of fundamental biological processes, correlations between variation in mushroom body morphology and cocaine preference in the fly brain raise the possibility that subtle variations in neural circuitry in the human brain could contribute to risk for cocaine use disorder.

567F **Characterizing the impact of mating on proboscis extension response in Female *Drosophila melanogaster*** Rebecca Fakunle[1], Sol Cabrera[1], Genevieve Bell[2] [1]Centre College, [2]Neuroscience, Centre College

The female *Drosophila Melanogaste*r undergoes a complete behavioral shift post mating that is characterized by decreased receptivity, increased appetite, and a preference for salt and yeast foods. This post mating shift is the result of a seminal fluid protein, Sex Peptide, that activates a neural circuit in females, altering their behavior. Previous studies have found that along with broad changes in behavior, a female's reproductive state can influence chemosensory perception. However, the mechanisms that modulate mating-induced changes to chemosensory processing remain largely unknown. Thus, the impact of mating on proboscis extension response (PER) in female *D. melanogaster* was studied. We performed PER assays to assess the detection of the four basic tastes (sweet, sour, salt, and bitter) in virgin and mated females. Flies were exposed to serial dilutions of each tastant at millimolar concentrations, following the method of limits psychophysical approach to generate a dose-response curve for each tastant. Preliminary data has been promising, showing an overall increase in detection for all basic tastants in mated over virgin females. We are currently performing additional studies to increase sample sizes across groups. Collectively, this study will allow for further elucidation of the neural circuitry that underlie mating induced changes to chemosensory systems. By studying these changes, we can better understand the principles of neuromodulation—how sensory systems alter their activity based on physiological state.

568F **Mechanisms of sleep-associated huddling behavior in *Drosophila melanogaster*** Seth R Odell, Ritika Joshi, Matthew Meiselman School of Life Sciences, University of Nevada, Las Vegas

The formation of groups can provide many benefits to individuals within the group, including increased situational awareness due to overlapping perceptions. Although not thought of as a herd animal or even a traditional social insect, *Drosophila melanogaster* form groups under certain circumstances. We sought to examine how grouping behavior (huddling) varies in free running flies over a continuous, extended period. We utilized a custom camera setup (LOOC) to capture these behaviors. Using the LOOC, we continuously recorded flies over a 24-hour period. We observed that the huddling behavior of flies changes over the course of a circadian day. This huddling behavior was highly anticorrelated with the overall activity and directly correlated with sleep. Sleep is a vulnerable state leaving animals with increased arousal thresholds, subsequently leading to increased predation risk. Some animals sleep in groups to pool their reduced awareness of such threats. To determine if sleep was causally linked with grouping, we assessed huddling in sleep deprived flies. Further, we administered depressants or stimulants and assessed whether huddling timing and frequency were affected. Sleep reduces arousal threshold, which may necessitate grouping to improve situational awareness among the group's individuals. Further, communication through sensory cues (visual, olfactory, auditory, etc.) is vital to social behaviors. To examine the role of these sensory cues in huddling, we utilized various sensory null mutants to determine the importance of that sensory modality to this sleep huddling behavior. Long term, our identification of modalities that are critical for huddling will lead to the neural circuits of huddling behavior, which may offer insight into the neurobiology of an important ecological phenomenon, herding.

569F **A flashing success: resolving the effects of neuromodulators at the active zone level** Jocelyn C. Bransford[1], Kiel G. Ormerod[2] [1]Biology, Middle Tennessee State University, [2]Middle Tennessee State University

Animal behavior is generated in the central nervous system via neural pathways, which dictate motor output to control the activity of muscles in the periphery. One of the main mechanisms exploited by animals to achieve precise orchestration of neural circuitry and the governance of behavior through effector cells is through the coordinated release of neuromodulators. These neuromodulators can be released locally at chemical synapses, or into circulation to alter synaptic transmission. There have been over 100 identified in the human CNS, and up to 100-200 within various arthropod species. Physiological functions have been characterized for many neuromodulatory substances, but a substantial gap in our understanding of their mechanism of cellular action remains. The neuromuscular junction (NMJ) of *Drosophila melanogaster* is an excellent system for characterizing the formation, function, and plasticity of a model synapse. It has been used in investigations of neuromodulation for several decades due to its ease of manipulation and access for electrophysiological recordings, immunolabeling, and live imaging. In addition, high-throughput, high-resolution optical approaches have been optimized to label and examine subcellular structures, cells, and neural circuits within the fly model. Our lab has refined these approaches and many others to characterize the effects, pathways, and genetic underpinnings of many different neuropeptides, including FMRFa2, octopamine/tyramine26, proctolin27, and tachykinin28, and more. Here, we build upon the synaptic calcium sensor GCaMP to examine the effects of modulators on individual synapses and active zones. Combining GCaMP imaging with molecular approaches available in *Drosophila*, we dynamically examine how cellular factors, neuronal activity, and modulatory substances affect the development and function of synapses.

570F **Glutamate signaling controls dormancy-associated quiescence** Jilian Morejon[1], Seth Odell[1], Ellie Vincent[1], Matthew R. Meiselman[2] [1]School of Life Sciences, University of Nevada Las Vegas, [2]University of Nevada Las Vegas

Animals have evolved a variety of strategies which help them cope with adverse conditions. Many insects, including Drosophila melanogaster, respond to sustained cold by entering a state of behavioral and reproductive quiescence, known colloquially as dormancy. As temperature information is predominately sensed and transduced through the nervous system, we sought to understand the neurochemical basis that underlies dormancy. Here, we characterized the declined activity, and elevated sleep observed in dormancy. We found sleep is non-homeostatic in nature, as recovery from dormancy was not associated with a sleep rebound and took several days to normalize. To determine if sleep and dormancy have unique or shared neurochemical bases, we fed flies sleep-repressing or stimulating drugs and assessed activity and egg production. We found that consumption of only depressants and stimulants that target the glutamatergic system (MK-801 and phenobarbital) were able to modulate dormancy-associated sleep and, surprisingly, egg production as well. Further, activation of glutamatergic neurons or NMDAR-1-expressing neurons put flies in a dormancy-like state in warm temperatures. RNAi for the glutamate transporter *VGlut* resulted in increased egg production and activity. This work suggests a novel pathway for dormancy and may offer insight into the diversity and complexity of sleep regulation.

571F **Neural Modulators of Metabolism in *Drosophila melanogaster*** Victoria Campos, Logan Kazimer, Katelyn Niswonger, Brandon Polimeni, Emily Timmins, Ellie Vincent, Matthew Meiselman University of Nevada Las Vegas

Outside of the tolerable bounds of environmental factors, animals preserve energy by limiting growth and attenuating reproduction. The role of the nervous system in perceiving these factors and executing state changes remains poorly understood. Here, we leveraged the genetic tools available in Drosophila melanogaster to investigate the neural regulation of metabolism and reproduction. Using the Gal4-UAS system, we screened 173 Gal4 lines for those that attenuated anabolism and egg production when thermogenically activated, and used the 15 lines which reduced egg production the most to generate 168 Split-Gal4 lines, and further identified specific neuron subsets whose activation disrupts anabolism, measured by egg production. We identified several unique neuron subsets which we are currently in the process of characterizing, notably including neurons housed in the Johnston's organs, primary sensory neurons for temperature entrainment. This study will offer insight into neural circuits by which the brain integrates perception into metabolic state.

572F **Exploring the Behavioral Effects of the Probiotic *Lactiplantibacillus plantarum* in *Drosophila* and the Role of the Intestinal Alpha-4 Nicotinic Acetylcholine Receptor** Geraldine M. Ortiz Sosa, Melanie Reinoso Arnaldi, Imilce De Los Angeles Rodriguez Fernandez Biology, University of Puerto Rico, Rio Piedras

The gut-microbiota-brain axis is a complex communication network that connects the brain, the gut, and its resident microbiota. While this axis is vital for maintaining gastrointestinal homeostasis, it influences behavior and mental activity. Besides the nervous system, neurotransmitters can also be produced by the intestinal epithelium and gut bacteria. Of interest is *Lactiplantibacillus plantarum* (*L. plantarum*), a probiotic and commensal bacteria that produces acetylcholine (ACh). Studies in *Drosophila* have shown that ACh receptors are expressed by gut enterocyte cells and ACh can arise from gut enteroendocrine cells and anti-inflammatory recovery-specific cholinergic enteric neurons. This project focuses on exploring the role of ACh signaling in the *Drosophila* gut and assesses whether *L. plantarum* could serve as a significant source of ACh, as well as test its potential to modify different behaviors. To do this, we generated transgenic flies in which we knocked down nicotinic acetylcholine receptor alpha 4 (nAchRa4) or control using RNAi in the gut for 11 days during adulthood. Then we administered *L. plantarum* (strain LpWF) or mock treatment and measured the climbing ability of treated control and transgenic flies 1, 3, 24, and 120 hours after treatment. Our data suggest that motor function in young *Drosophila* adults is altered by the administration of *L. plantarum* strain WF at 3 hours, with recovery observed after 24 hours. However, this effect is not observed when nAchRa4 is knocked down which suggests that this LpWF-mediated effect may require AchRα4 expression in the gut. We also developed a new protocol to detect neurotransmitters produced by bacteria biofilms growing on glass slides. Our preliminary immunostaining results suggest ACh could be detected in all bacteria biofilms tested: *L. plantarum* (two stains LpWF and Lp39) and E. coli. Our results suggest that the changes in behaviors observed with *L. plantarum* administration may require intestinal ACh signaling and that *L. plantarum* could produce ACh. We aim to characterize the molecular mechanism by which LpWF modifies climbing behavior and the role of acetylcholine signaling in this process.

573S **Roundup Exposure Decreases Activity of *Drosophila melanogaster*** Katherine Bartels[1], Becky Talyn[2] [1]Biology, California State University, [2]College of Natural Sciences, California State University

Glyphosate-based herbicides (GBHs) kill weeds and leaves around produce like grains, legumes, and sugar cane. GBHs elicit great concern because of their environmental impact in agro-ecosystems, and because residues on food may affect human health. This study examines whether Roundup Super Concentrate (RSC), a GBH, affects activity levels, using *Drosophila melanogaster*. We exposed flies to 10, 5, or 2g/L RSC, 10g/L glyphosate, 2g/L polyethoxylated tallow amine (POEA, the surfactant used in RSC), a combination of 5g/L glyphosate and 2g/L POEA (the relative proportions in RSC), or control medium before using a *Drosophila* Activity Monitor to record how often the flies cross a light beam in each 24-hour trial. We tested 20 individual flies of each age, 3, 5, and 7 days, for both males and females. In addition, some flies were exposed to the 10, 5, and 2g/L RSC treatment for two days before being released to the control medium to assess whether the effects of exposure were permanent or whether their activity levels could recover. We found that flies exposed to RSC exhibited less activity, especially females and especially at higher concentrations, while released flies recovered only partially. Exposure to glyphosate and POEA, alone or in combination, does not affect their activity levels, indicating that other ingredients in the formulation are responsible for this effect. Since patent laws in the US allow companies to maintain proprietary formulations without disclosing non-active ingredients, it is difficult to determine the specific cause of toxicity. Given that environmental contamination will occur based on entire formulations, however, that is the most relevant source of exposure for exploring environmental impacts. Reduction in activity is important for animal fitness because it impacts the ability to find food, find mates, and avoid predators. In our current work, we are assaying the protein content of *Drosophila* exposed to RSC, to determine if the reduction in activity results from the quantity of food ingested or its quality (glyphosate acts as an antimicrobial, which may influence nutrient quality of food) when the food source contains RSC.

574S **Orb2's ability to form amyloid instead of its specific structure governs long-lived memory** Kaili Li[1], Ruhar Singh[2], Consuelo Perez Sanchez[3], Paulo Leal[1], Sunny Sharma[4], Ruben Hervas Milan[5], Łukasz Joachimiak[2], Kausik Si[1] [1]Stowers Institute for Medical Research, [2]UT Southwestern Medical Center, [3]Janelia Research Campus, [4]The University of Tennessee Health Science Center, [5]School of Biomedical Sciences, Hong Kong University

Amyloids are present broadly in nature under both diseased and normal conditions in vivo. Amyloids of different primary sequences possess common defining features such as a cross-beta-sheet core, self-templating ability, and resistance to denaturant or detergent. Nevertheless, these amyloids can have distinctive structures, molecular activities, and biological functions. The Orb2 protein forms amyloid in the Drosophila brain, which switches the Orb2 monomer's function as a translational repressor to a translational activator. This conformational switch is essential for Orb2 to regulate memory consolidation and persistence. Here, we showed that altering the amyloid-forming domain of Orb2 has profound consequences on memory formation or persistence. Flies carrying mutations that accelerate amyloid formation display a deficit in the learning process, but memory persists once formed. On the other hand, flies with mutations impairing amyloid formation have comparably normal learning abilities, but the formed memory fails to persist. In addition, swapping the Orb2 prion-like domain with other amyloid-forming domains, such as those in distantly related Orb2 homologs or the prion-like domain of yeast Sup35, maintains normal Orb2 function in memory regulation. These results indicate that a specific structure of the Orb2 amyloid is not needed for its normal function. Consistent with this proposition, we showed that a point mutation in Sup35 that decreases its ability to form amyloid also leads to a deficit in memory persistence when placed in an Orb2 context in vivo. Our study thus suggests that the ability of Orb2 to form amyloid, instead of a specific amyloid structure, is essential for memory formation and persistence. It raises the possibility that by controlling or modifying the amyloid formation process of Orb2, flies control learning potentials or memory durations toward different objects.

575S **Males use color vision to define the anterior-posterior body axis of courted females** Christian J Monroy Hernandez[1], Ross M McKinney[1], Iris Chin[2], Yehuda Ben-Shahar[1] [1]Washington University in St. Louis, [2]Gladstone Institute

Courtship is a type of social interaction exhibited by many organisms, frequently as ritualistic displays composed of spatial and temporal transitions between discrete behavioral elements. However, for most species the underlying sensory signals and neural circuits driving the spatial and temporal aspects of these displays remain unknown. In *Drosophila melanogaster*, males engage in courtship by displaying stereotyped behaviors such as tapping, orienting and scissoring. One of the main sensory inputs influencing courtship is visual stimulus. We recently identified that male flies exhibit distinct courtship elements at specific locations surrounding the female, seemingly relying on anatomical landmarks that define the female's anterior-posterior body axis. Machine-assisted high-resolution analysis of the male courtship ritual reveals that males use the eyes of courted females as a landmark for the anterior end of the female's body, which depends on their ability to distinguish red eyes from other female body parts. Furthermore, we present data that the ability of males to identify the female's eye location depends on R7 photoreceptor cells, which are tuned to color detection. Together, these data provide a simple neuroethological explanation for how males use female anatomy to regulate the spatial aspects of the courtship ritual, and provide an explanation, at least in part, for why flies have evolved color vision.

576S **The Role of Anchor gene in feeding in a *Drosophila* Knockdown model** Adekemi Sobukunola[1,2], Kamille Chestnut[1], Emmanuelle Palmieri[1], Fernando Vonhoff[1] [1]Department of Biological Sciences, University of Maryland Baltimore County, [2]LSAMP, University of Maryland Baltimore County

Feeding is an important part of human development. Adequate feeding is necessary for proper growth, homeostasis and development. Inability to control feeding could lead to health conditions such as obesity and eating disorders such as anorexia. Drosophila's rapid life cycle, mass reproduction ability, similar human genomes and capability to be given diets that are carefully regulated makes it a great model for studying feeding related behavior. Insulin producing cells(IPCs) are responsible for producing and releasing the hormone insulin, which helps cells absorb glucose and controls blood sugar levels. Behaviorally, therefore, IPCs are involved in feeding and circadian rhythm as circadian signals can influence how much food is consumed by an organism. Here, we identify an understudied protein, anchor as being likely expressed in Drosophila IPCs and involved in feeding and circadian behaviors. Anchor protein was previously shown to be involved in Drosophila wing development, and it is suspected to be involved in signaling pathways associated with cell growth. However, its behavioral role has never been characterized, and despite expression in the brain, its neurological function is unknown. We investigated the role of Anchor in feeding behavior, using an anchor knockdown model in Drosophila. We hypothesized that knocking down Anchor gene in flies will impact their feeding behavior as well as their circadian rhythm. We used the Capillary Feeding Assay to measure the quantity of food consumed by Drosophila over a 24 hour period and compared the amount consumed by genetic control groups to the anchor knockdown group. We also employed the use of a flyPAD, (fly Proboscis and Activity Detector) that detects the physical interaction between individual Drosophila and food using capacitive-based measures. This flyPAD was used to measure the amount of food consumed by Drosophila in a single sip. Preliminary data show that flies with the anchor gene knocked down in them tend to consume less with time compared to the controls. We also measured circadian rhythm, using a 24-hour automatic tracking assay, the Drosophila Activity Monitor (DAM). Preliminary results show that anchor knockdown animals have reduced circadian entrainment and lower overall locomotion compared to genetic control groups. The future directions of the study will focus on insulin neuronal-specific knockdown experiments to examine how reduced insulin signaling affects the nervous system.

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577S **The Role of Anchor in Feeding in a Drosophila Knockdown Model** Adekemi Sobukunola[1,2], Kamille Chestnut[1], Emmanuelle Palmieri[1], Fernando Vonhoff[1] [1]Department of Biology, University of Maryland, Baltimore County, [2]LSAMP, University of Maryland, Baltimore County

Feeding is an important part of human development. Adequate feeding is necessary for proper growth, homeostasis and development. Inability to control feeding could lead to health conditions such as obesity and eating disorders such as anorexia. Drosophila's rapid life cycle, mass reproduction ability, conserved molecular pathways and capability to respond to diets that are carefully regulated makes it a great model for studying feeding related behavior. Insulin producing cells (IPCs) are responsible for producing and releasing the hormone insulin, which helps cells absorb glucose and controls blood sugar levels. Behaviorally, therefore, IPCs are involved in feeding and circadian rhythm as circadian signals can influence how much food is consumed by an organism. Here, we identify an understudied protein, Anchor, as being likely expressed in Drosophila IPCs and involved in feeding and circadian behaviors. Anchor protein was previously shown to be involved in Drosophila wing development, and it is suspected to be involved in signaling pathways associated with cell growth. However, its role in animal behavior has never been characterized, and despite expression in the brain, its neuronal function is unknown. We investigated the role of Anchor in feeding behavior, using a knockdown model in Drosophila. We hypothesized that knocking down anchor in flies will impact their feeding behavior as well as their circadian rhythm. We used the Capillary Feeding Assay to measure the quantity of food consumed by flies over a 24 hour period and compared the amount consumed by genetic control groups to the anchor knockdown group. We also employed the use of a flyPAD, (fly Proboscis and Activity Detector) that detects the physical interaction between individual flies and food using capacitive-based measurements. This flyPAD was used to measure the amount of food consumed by flies in a single sip. Preliminary data show that flies with the anchor gene knocked down in them tend to consume less food with time compared to controls. We also measured circadian rhythm, using a 24-hour automatic tracking assay, the Drosophila Activity Monitor (DAM). Preliminary results show that anchor knockdown animals have reduced circadian entrainment and lower overall locomotion compared to the genetic control group.The future directions of the study will focus on insulin neuronal-specific knockdown experiments to examine how reduced insulin signaling affects the nervous system.

This research was partially funded by the USM LSAMP program, supported by NSF LSAMP Award #2207374

578S **Arc1 functions in insulin producing cells to regulate sleep** Kaitlyn Pamplona, Jessa Maglio, Katherine Darrow, Elizabeth Brown Biological Sciences, Florida State University

Neural regulation of sleep and metabolic homeostasis are critical in many aspects of human health. Despite extensive epidemiological evidence linking sleep dysregulation with obesity, diabetes, and metabolic syndrome, little is known about the neural and molecular basis for the integration of sleep and metabolic function. The gene Activity regulated cytoskeleton protein 1 (Arc1) has been linked to synaptic plasticity and metabolic function, which play crucial roles in sleep regulation and raise the possibility that it functions to control these processes. Here we characterize the effects of Arc1 on sleep duration. Flies lacking Arc1 significantly increase sleep duration by increasing the length of individual sleep episodes, raising the possibility that loss of Arc1 promotes deep sleep. The effects of Arc1 on sleep duration can be localized to neurons expressing the Drosophila insulin-like peptide (Dilp2), which has been previously implicated in the metabolic regulation of sleep depth. Silencing expression of Arc1 in these neurons significantly increases sleep duration, phenocopying Arc1 mutants, while overexpression of Arc1 significantly decreased sleep duration. We also find that Arc1 neurons are acutely required for increased sleep duration. A key hallmark of sleep depth in mammals and flies is a reduction in metabolic rate during sleep. We are currently investigating whether Arc1 functions in Dilp2-expressing neurons to regulate metabolic rate during sleep. Together, these findings will shed light on the role of Arc1 function in insulin-producing cells in sleep quality in Drosophila. Overall, this work contributes to our understanding of Arc1 function in the regulation of sleep.

579S **Temporal cohort number, neural composition, and connectivity vary along the *Drosophila* larval body axis** Deeptha Vasudevan, Yi-wen Wang, Hannah Carr, Elaine Kushkowski, Sean Corcoran, Chris Wreden, Ellie Heckscher Molecular Genetics and Cell Biology, University of Chicago

Neural circuits are the fundamental computational units of the nervous system, and neural stem cell lineages are the fundamental developmental units of the nervous system. How these units map onto each other is a critical constraint on CNS function. The developing Drosophila nerve cord has emerged as a powerful model for studying lineage-based circuit assembly. Here, lineages are organized into temporal cohorts, which are sets of similar neurons born within a tight time window, and connectomic analysis shows that neurons of a temporal cohort have similar circuit-level connectivity. A major limitation has been the focus on a single representative segment, which is problematic because the body and nervous system comprise multiple segments organized into axially diverse regions, each with distinctive behaviors and circuits. How variation in stem cell lineage development impacts circuit configuration is unknown. Here, using the Drosophila NB3-3 lineage at different axial levels as a model, we determine the variation in cell biological decisions made by the lineage and examine its impact on temporal cohorts. We combined lineage tracing, manipulation of cell death pathways, marker gene analysis, and anterograde circuit tracing from somatosensory neurons. Depending on region, temporal cohort number is modulated by stem cell proliferation. The number and molecular identities of neurons within a temporal cohort are tuned by various cell biological mechanisms, all acting post-mitotically. Temporal cohorts can be mapped into similar or different circuits. Overall, this study provides new fundamental insight into how diverse circuits emerge from a constrained pool of stem cells in vivo.

580S **Genetic Analysis of the Moonwalking Descending Neuron (MDN) Circuit in Drosophila melanogaster** Zhehao Zhu, Matthew Clark Bucknell University

The moonwalking descending neuron (MDN) circuit, a part of the neural circuits of Drosophila melanogaster, is responsible for backward locomotion. Our experiment explores the function and how transcription factors (TFs) and Cell-surface molecules (CSM) contribute to the neuronal identities and connectivity in the MDN circuit. However, how the MDN circuit works with different genes is unclear. Our results identify several genotypes that significantly modulate MDN-driven behavior. Optogenetic activation of MDNs in these genotypes reveals varying degrees of forward and backward locomotion, indicating differential effects on MDN circuit integrity. For the experiments that show a certain level of forward locomotion, they are “hits”, which would proceed to the next step. Our experiment showed a great amount of GAL4/UAS lines that express a “hit”, demonstrating how each Gal4 driver affects the adults differently. With the genes that show “hits”, more research can be conducted on certain genes to give a pattern of the neuron circuit, not only in drosophila but in other organisms as well. Also, with further assay, the understanding of the neuron circuit can lead to the development of technology in the medical field, which helps with the improvement of therapies that help fix the damage to the neuron circuit and further research on brain development and function.

581S **Development of a Single Fly Aversive Learning Assay suitable for use in the classroom.** Amanda Crocker Neuroscience, Middlebury College

Here we describe the development of an adaptable assay to train 5 flies individually with electric shock and odor. Each individual assay can be built at a cost that is reasonable for an undergraduate institution and can be scaled up. This can be used in a classroom lab setting where each group can train 5 flies at a time, collect and analyze data. The assay relies on video tracking, allowing for data analysis to be done offline. The chambers are adaptable to test for electric shock avoidance, light preference, odor preference, heat preference and courtship assays can be run in them. This assay allows for unique opportunities for students in a classroom setting to learn about fly genetics, discover how mutations impact behavior and to grasp how messy behavior actual is to quantify and study.

582S **Sirt6 regulates learning and memory in *Drosophila melanogaster*** Prema Singaravel, Samira Xhaferi, Roja Sharma, Jackson Taylor Center for Gene Regulation in Health and Disease, Department of Biological, Geological and Environmental Sciences, Cleveland State University

Sirt6 is an NAD^+-dependent protein deacetylase which plays major roles in DNA repair, glucose and lipid metabolism, and immune regulation. Despite extensive study in a variety of tissues, little is known about the specific function of Sirt6 in the brain, particularly in the context of learning and memory. Here, we examined the role of Sirt6 in learning and memory, using *Drosophila melanogaster* as a model system. Using an established model of aversive phototaxic suppression, we found that flies lacking a functional copy of Sirt6 (*Sirt6* $^{-/-}$ flies) have significantly impaired learning and short-term memory. Neuronal-specific knockdown of Sirt6 also impairs learning and memory, suggesting Sirt6 activity in the brain is required for these functions. Sirt6 knockdown and deletion flies also have increased H3K9 acetylation in head tissue, an epigenetic mark associated with active transcription. Transcriptomic profiling revealed that *Sirt6* $^{-/-}$ flies have altered expression of genes involved in neuropeptide signaling and glycerophospholipid metabolism in head tissue, suggesting possible mechanisms by which Sirt6 regulates learning and memory.

583S **Role of Robo3 in Specific Hemilineages during Pupal Development** Samuel D Herman, Erin A Beck, Haluk Lacin Biological and Biomedical Sciences, University of Missouri-Kansas City

Drosophila melanogaster goes through two phases of neurogenesis. The embryonic phase establishes the larval central nervous system (CNS); the postembryonic phase, from 2nd instar to late pupal stage, establishes the adult CNS. Axon guidance molecules have been extensively researched in the embryonic stage for their role in establishing the larval CNS, but how they organize the adult CNS is not well-understood.

The ventral nerve cord (VNC) of *Drosophila* is analogous to the mammalian spinal cord, performing reflexive motions as simple as kneeling and as complex as take-off. Hemilineages are the fundamental unit of the VNC; they are defined as neurons that arise from the same neuroblast, express a set of similar transcription factors, and use the same neurotransmitters (glutamate, GABA, or acetylcholine). Hemilineages provide a framework for understanding circuit formation during development. We lack clarity for how hemilineages express axon guidance molecules during the pupal stage to form the neuronal circuitry present in a healthy adult. The Roundabout (Robo) family mediates axon repulsion from the midline and organizes axons into lanes which differ in their distance from the VNC midline. To understand the role of axon guidance molecules in the pupal stage, I analyzed Robo3 expression using a hemilineage-based approach.

I first used neurotransmitter-specific driver lines to visualize whether there is an extensive overlap between robo3 expression and cellular processes of GABAergic, glutamatergic, and cholinergic neurons, finding extensive Robo3 localization in glutamatergic neurons. To identify which glutamatergic hemilineages show Robo3 expression, I utilized our lab's split-Gal4 library, which genetically and specifically labels individual hemilineages. Of the hemilineages I screened, both 14A & 9A displayed extensive overlap with Robo3 protein. Then, to understand the role Robo3 plays during pupal development, I am currently performing a hemilineage-specific knockdown of Robo3 to identify whether Robo3 is required for proper axonal architecture of 9A and 14A neurons.

584S **Exploring alcohol sensitivity and tolerance in *Drosophila*: The crucial role of Tip60 in ventrolateral neurons (LNv)** Angelica M Crespo-Rodriguez, Christian D Del Valle-Colon, Airined Montes-Mercado, Alfredo Ghezzi Department fo Biology, University of Puerto Rico, Rio Piedras Campus

Alcohol Use Disorder (AUD) develops when someone struggles to control their drinking and needs more alcohol to achieve the same effects. Consequently, alcohol consumption has the potential to become addictive and can cause a series of neuroadaptations such as tolerance, dependency, and withdrawal symptoms. These neuroadaptations can be partially explained by the activation of epigenetic mechanisms that influence multiple biological and behavioral processes in the organism. However, these mechanisms remain incompletely explored, leaving significant gaps in our understanding of their role in regulating these processes. We specifically examined the role of Tip60, a histone acetyltransferase (HAT), which we believe to have an effect in alcohol tolerance. There are also indications that alcohol affects sleep/wake cycles of various organisms as a way of the nervous system adapting to its disruptions. Therefore, we will also be studying the ventrolateral neurons (LNv), who produce the neuropeptide pigment-dispersing factor (pdf) in order to stabilize sleep/wake cycles. Using the *Drosophila melanogaster* model, we sought to identify the behavior of the fly after being exposed to ethanol, four hours (Zt_4) after their light/dark sleep cycle. We hypothesize that when Tip60 is knocked down, we will be able to see that Zt_4 after the light turns on, the flies may be more sensitive to alcohol but will develop tolerance the same as after eight hours (Zt_8). Previously, in our lab, this procedure was done with the flies being exposed to alcohol Zt_8 after the light turns on, resulting in the flies being more resistant when exposed just once, and then developing tolerance after their second exposure. To test this new hypothesis, we will use the UAS-GAL4 system to knock down Tip60 expression in the nervous system of female flies. The flies will be separated by using pdf-gal4<UAS-Luc-RNAi as the control group during the experiments, and pdf-gal4<UAS-Tip60-RNAi will function as the experimental group. They will be exposed to water and 50% ethanol vapor on the first day, and then after 24 hours they will all be exposed to ethanol. With this assay, we expect to determine whether tolerance development differs when flies are exposed to Zt_4 into their regular light/dark cycle, compared to previous studies conducted in our lab at Zt_8. In the future, we plan to extend these procedures to additional Zt points to explore whether distinct effects emerge at different times within the light/dark cycle.

585S **Genetic Screen for Proprioceptor Morphology and Function.** Dorian J Dale, Madison Bouggess, Liping He, W. Dan Tracey Indiana University

Proprioception is the sensory process encoding the body position and movement of an organism. Proprioceptors are specialized sensory neurons responsible for sensing proprioception. The class I md neurons of *Drosophila melanogaster* larvae, termed ddaE, ddaD and vpda, function in proprioception. The dendrites of the class I md neurons tile each hemi-segment of the larval body wall and have been shown to undergo deformations as the peristaltic muscle contractions travel along the longitudinal axis of the body during crawling. Mutations in *Drosophila transmembrane channel-like (Tmc)* prevent Ca^{2+} responses of the ddaE and ddaD cells during movement, indicating that TMC is required for neuronal activation during crawling. Notably, ddaE responds robustly to forward crawling and not reverse crawling, while ddaD responds to reverse crawling but not forward crawling. This directional selectivity is a common feature of TMC expressing cells. Mammalian hair cells of the inner ear also display directionally selective activity and express TMC, which localizes to individual stereocilium and is required for activation. TMC relies on additional factors for its directionally selective activation and mutation of these factors leads to deafness in humans and other vertebrates. We are testing the hypothesis that TMC's role in proprioception is dependent on additional co-factors such as those that are important in mammalian hearing. Preliminary behavioral analysis using an olfactory navigation assay has revealed that TMC mutant larvae require 3x the time to reach an attractive odor source as control larvae. Our analysis suggests that much of the delay in reaching the odor source appears to be occurring in the early stages of navigation, potentially due to an impairment in the association of head casting with olfactory inputs. Interestingly, once engaged in crawling towards an odor source, TMC mutant larvae crawl at the same speed as controls, suggesting TMC mutants do not simply have a crawling defect. Additionally, we have conducted an imaging based genetic screen using RNAi to knockdown genes enriched in larval proprioceptors. Preliminary results demonstrate a possible conserved interaction between TMC and cadherins, potentially critical for neuronal function and proprioceptive behavior. Future directions for this project are a detailed characterization of the behavior, exploring whether these proteins interact by colocalization imaging and calcium imaging of the neurons during crawling to determine whether TMC-dependent activation is disrupted in animals lacking relevant cadherin genes.

586S **miRNA-Mediated Regulation of VGLUT Expression in VPM Neurons Drives Sexually Dimorphic Feeding Behavior** Solange C Holman[1], Faith You[2], Liam Dunning[3], Erin Szalda-Petree[4], Ronald S Stowers[5], Elizabeth Catudio-Garrett[1], Sarah J Certel[1] [1]Biological Sciences, University of Montana, [2]Hellgate High School, [3]Sentinel High School, [4]Applied Mathematics, University of Washington, [5]Microbiology and Cell Biology, Montana State University

The neurotransmitter glutamate is essential for the proper functioning of the central nervous system. In presynaptic neurons, glutamate is packaged by vesicular glutamate transporters (VGLUTs) into synaptic vesicles, followed by release into the synaptic cleft by stimulation. The expression level of VGLUTs in a synaptic vesicle is a key determinant of the quantal size of glutamatergic transmission, making regulation of VGLUTs essential. Recently, we found that VGLUT levels increase in the glutamatergic+/octopaminergic+ VPM1 and VPM4 co-transmitting neurons in the adult fly brain as the organism ages, with males exhibiting a greater increase than females. This finding has led us to investigate the factors regulating VGLUT levels and determine the behaviors controlled by glutamate signaling from VPM1 and VPM4 neurons. MicroRNAs (miRNAs), particularly miR-1000, have been shown to target the 3' untranslated region (UTR) of VGLUT, providing an additional layer of post-transcriptional control over glutamate release. As expected, we confirmed that expressing a miR-1000 sponge in VPM1 neurons resulted in higher VGLUT expression levels.

In this study, we determined that within VPM1 and VPM4 neurons, miR-1000 and miR-1008 are critical for regulating food consumption in males but not females. Specifically, driving the expression of a miR-1008 sponge in VPM4 neurons increased VGLUT levels, which led to reduced food consumption in males, while female feeding behavior remained unaffected. In contrast, the expression of the miR-1008 sponge in VPM1 neurons resulted in increased food consumption in males, again without affecting female feeding behavior. Using a bioinformatics approach, we identified four potential binding sites for miR-1008 in the 3' UTR of VGLUT. In ongoing studies, we are asking whether the sexually dimorphic differences in VGLUT-regulated feeding behavior are influenced by the male-specific isoform of fruitless (FruM), as VPM1 glutamatergic+/octopaminergic+ co-transmitting neurons express FruM.

Determining how miRNAs modulate VGLUT expression in the co-transmitting VPM1 and VPM4 neurons will provide important insights into the regulation of glutamatergic signaling maintaining neural, behavioral homeostasis and the molecular mechanisms regulating feeding behavior. This work will also shed light on the complex post-transcriptional regulation enabling the nervous system to dynamically adjust neurotransmission in response to internal and environmental cues.

587S **Glutamate signaling controls dormancy-associated quiescence** Jilian Morejon[1], Seth Odell[2], Ellie Vincent[2], Matthew Meiselman[2] [1]Life Sciences, University of Nevada, Las Vegas, [2]University of Nevada, Las Vegas

Animals have evolved a variety of strategies which help them cope with adverse conditions. Many insects, including *Drosophila melanogaster*, respond to sustained cold by entering a state of behavioral and reproductive quiescence, known colloquially as dormancy. As temperature information is predominately sensed and transduced through the nervous system, we sought to understand the neurochemical basis that underlies dormancy. Here, we characterized the declined activity, and elevated sleep observed in dormancy. We found sleep is non-homeostatic in nature, as recovery from dormancy was not associated with a sleep rebound and took several days to normalize. To determine if sleep and dormancy have unique or shared neurochemical bases, we fed flies sleep-repressing or stimulating drugs and assessed activity and egg production. We found that consumption of only depressants and stimulants that target the glutamatergic system (MK-801 and phenobarbital) were able to modulate dormancy-associated sleep and, surprisingly, egg production as well. Further, activation of glutamatergic neurons or NMDAR-1-expressing neurons put flies in a dormancy-like state in warm temperatures. RNAi for the glutamate transporter *VGlut* resulted in increased egg production and activity. This work suggests a novel pathway for dormancy and may offer insight into the diversity and complexity of sleep regulation.

588T **Unraveling the Molecular Clock of Neurogenesis in Drosophila Medulla Using Live Imaging** Khaled Ben El Kadhi[1], Claude Desplan[2] [1]New York University Abu Dhabi, [2]New York University

The Drosophila compound eye consists of 800 unit eyes, each containing 8 photoreceptors (PRs). These PRs transmit visual information to the optic lobe's visual processing centers: the lamina, medulla, and lobula. Among these, the medulla is the most complex, comprising approximately 40,000 neurons derived from 800* medulla neuroblasts (NBs) originating in the larval outer proliferation center (OPC). The OPC's NBs divide asymmetrically, self-renewing and producing ganglion mother cells (GMCs), which generate two distinct medulla neurons. Temporal transcription factors (tTFs) have been shown to sequentially regulate neuroblast differentiation, with early studies identifying six key tTFs that drive neuronal diversity. Recent single-cell sequencing efforts in our lab have expanded this list to 11 tTFs. However, while the tTF cascade is understood, key aspects remain unclear, including the precise timing, duration, and transitions between tTF expression states.

To address these gaps, we have developed a primary culture system for NBs to investigate the molecular clock driving the tTF cascade through live-imaging (L-I). By using CRISPR-Cas9-mediated endogenous tagging of tTFs with distinct fluorescent proteins, we quantified the duration of competence windows, the number of cell divisions, and the timing of transitions between tTFs. This approach provides dynamic, real-time insights into how tTFs govern neuroblast differentiation. Ultimately, this research aims to inform strategies for programming naïve neural stem cells to generate specific types of neurons for potential use in cell replacement therapies.

589T **The temporal transcription factor Hunchback functions post-mitotically in descending neurons** Kristen Lee, Chris Q Doe Institute of Neuroscience

During development, *Drosophila* neuronal stem cells divide asymmetrically to generate neuronal progeny. A singular stem cell, referred to as a neuroblast, expresses transcription factors in distinct temporal windows to generate unique progeny. The first temporal transcription factor in this cascade is Hunchback (Hb), which is well-characterized in ventral nerve cord neurons but under-studied in central brain neurons. To fill this knowledge gap, we assayed Hb function in two central brain neuron types – the Moonwalker Descending Neuron (MDN) and Pair1. Both of these neurons express Hb, are descending neurons that persist into adulthood, and function in a locomotor circuit. In the ventral nerve cord, Hb can be either highly or lowly expressed in neuronal progeny. We assayed Hb expression in MDN and Pair1, finding that Hb expression is consistently higher in MDN compared to Pair1. Previous studies have shown that Hb does not function post-mitotically in ventral nerve cord neurons. Interestingly, we found that Hb does function post-mitotically in MDN and Pair1, but its function is different between these two neuron types. In the larvae, Hb regulates MDN, but not Pair1, morphology. Specifically, Hb knockdown in MDN leads to an increase in dendritic and axonal projections. However, in Pair1, Hb is required to inhibit the formation of aberrant larval synapses. Hb is also required in both MDN and Pair1 for normal locomotor behavioral output. During metamorphosis, MDN and Pair1 continuously express Hb while pruning and regrowing their neurites, forming their adult morphologies and circuits. Next, we knocked down Hb in MDN and Pair1 during metamorphosis and assayed these neurons during adulthood. Although the studies investigating Pair1 are ongoing, we have found that Hb is still required for normal morphology and behavior in adult MDNs. Interestingly, Hb knockdown in MDN leads to axon mistargeting. Taken together, these data suggest that Hb function is strikingly different between central brain and ventral nerve cord neurons. Within the central brain neurons assayed, the differences observed in Hb function may be due to whether Hb is highly or lowly expressed within the neurons. These studies provide novel insights into how early developmental programs within a stem cell can have long-term consequences within its mature neuronal progeny.

590T **Identification of *AANATL-7* expression using the UAS-GAL4 system in *D. melanogaster* to determine where histamine acetylation is occurring *in vivo*** Margaret Cubitt[1], Olivia Miller[2], Jared Lamp[3], Martin Burg[2] [1]Cell & Molecular Biology, Grand Valley State University, [2]Biomedical Sciences, Grand Valley State University, [3]Integrated Mass Spectrometry Unit, Michigan State University

The metabolism of histamine, a neurotransmitter used in Drosophila, involves synthesis of carcinine as an intermediate that can be converted back to histamine (1). Recently, it has been found that in addition to carcinine synthesis, histamine can be acetylated by the enzyme arylalkylamine N-acetyltransferase like-7, encoded by the *AANATL-7* gene (2). Mutations in the *AANATL-7* gene were generated and shown to disrupt the synthesis of N-acetylhistamine (NAH), determined through both immuno-fluorescent examination (3) and mass spectrometry analysis of histamine metabolites extracted from accessory glands that normally contain high levels of NAH (4). To understand where AANATL7 functions and how those tissues may be affected by *AANATL-7* mutations, the UAS-GAL4 heterologous expression system was employed. A 2.2 kbp *AANATL-7* promoter fragment was amplified and cloned into the NotI-BamHI sites of the pC3G4 plasmid (5) to generate the *AANATL7-GAL4*$^{2.2}$ promoter-GAL4 fusion. Injection of the resulting plasmid (pC3G4-AANATL7-GAL4$^{2.2}$) enabled the generation of transgenic flies that contained the P{*w*$^{+mC}$ AANATL7-GAL4$^{2.2}$} transgenic construct. After mapping the transgenic constructs, homozygous female P{*w*$^{+mC}$ *AANATL7-GAL4*$^{2.2}$} transformants were crossed to male flies bearing either P{*w*$^{+mC}$ UAS-2xEGFP} or P{*w*$^{+mC}$ 10XUAS-IVS-mCD8::GFP} transgenes. Accessory glands of F_1 male progeny were examined for GFP expression in live tissue. Results indicate that in all lines examined thus far (10+), males expressed GFP in the entire accessory gland. In addition, GFP fluorescence was present in a subset of cells in both the larval and adult central nervous system (CNS), indicating that AANATL7 may be acetylating histamine in the CNS. While the cells expressing AANATL7 in the larval CNS do not contain histamine-like immunoreactivity, they are labeled with the *elav* antibody but not the *repo* antibody, suggesting that the *AANATL-7* expressing cells in the CNS are neurons. Thus, we are focusing on the role of histamine acetylation in CNS function and male-specific behaviors to determine how disruption of NAH synthesis affects CNS or male accessory gland function in the *AANATL-7* mutants.

Citations:

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2. Dempsey et al (2015) doi: 10.1021/acs.biochem.5b00113.

3. Cruce et al (2024) 2024 TAGC Abstracts, 1750T.

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591T **Loss of drosophila GCN1 (CG17514) triggered the malfunction of adult neuronal circuits, which would be independent from the GCN1-GCN2 axis** Hidetaka Katow, Hyung Don Ryoo Cell Biology, NYU Grossman School of Medicine

General Control Nonderepressive 1 (GCN1) is known to activate General Control Nonderepressive 2 (GCN2) on the collided ribosome (disome) in starved cells. The ribosome collision arises when stall or stop of leading ribosomes due to the shortage of aminoacylated-tRNAs during the translation. Consequently, trailing ribosomes collide with the stalled or stopped one and form the disomes, which are non-functional in the translation. These unproductive ribosomes are required immediate clearance from the mRNA to maintain translation quality. GCN1 plays a crucial role in this process by binding to the disome, recruiting molecules such as GCN2, which triggers Integrated Stress Response pathway (ISR) or GTPBP1 and 2, which contribute to the Ribosome Quality Control (RQC). Thorough the interaction with these molecules, GCN1 can inhibit further collisions, remove disomes from mRNA and maintain translation fidelity. Although GCN1 is known to influence cellular viability through GCN2 and GTPBP1/2 regulations, its precise function under normal physiological conditions remains poorly understood.

To address this question, our lab generated a loss of function allele using CRISPR/Cas9, which is homozygote viable in the adult stages. In the regular condition, the mutant showed high mortality rate immediately post-eclosion. Survived flies did not walk around or climb the vial wall as control (w^{1118}) and $GCN2^{KO}$ (loss of function) flies did. To verify if these phenotypes are caused by an impairment of fly locomotion, the mutant was tested with the climbing assay. The results showed the significant defect of climbing ability and the bang-sensitive phenotype in the mutant, suggesting a neuronal basis for the observed deficits. Further, neuron-specific knockdown of GCN1 via elav-GAL4 phenocopied the mutant phenotypes, implying that GCN1 may play a role in neuronal function or circuit integrity. Supporting this idea, the result of heat-shock paralysis assay showed a severe seizure-like phenotype in the *gcn1* mutant.

Our findings suggest that Drosophila GCN1 is critical for maintaining neuronal function, with potential implications for seizure phenotypes in adult flies. These observations provide new insights into the role of GCN1 in neural regulation and highlight its possible contribution to seizure susceptibility.

592T **Involvement of Dlg1 in putative AIS protein composition in *Drosophila Melanogaster*** Vanessa Auld, Nat F Casson Zoology, University of British Columbia

The Axon Initial Segment (AIS), which is located adjacent to the axon hillock in myelinated and unmyelinated neurons, is the site of axon potential initiation. This region is identifiable by an increased expression of key AIS components such as scaffolding proteins and ion channels. The structure of the AIS is well characterized in vertebrate myelinating neurons, but it is not well understood in invertebrate and unmyelinated axons. Within *Drosophila melanogaster*, a putative AIS region is located at the boundary between the CNS and PNS on the Ventral Nerve Cord of 3rd instar larvae. We found the voltage-gated potassium channel Shal is localized with the voltage-gated sodium channel para in this region—as well as the scaffolding protein Ankyrin—in a manner similar to the distribution of proteins at the vertebrate AIS. Furthermore, we found that Basigin (Bsg) and the plasma membrane Ca+2 ATPase (PMCA) both co-localized to the AIS along with the MAGUK scaffolding protein Dlg1, suggesting a potential protein complex. Knockdown of Bsg leads to the loss of PMCA, but PMCA knockdown has no effect on Bsg localization. The effects of loss of Dlg1 on the Drosophila AIS protein complexes is unknown. Dlg1 has two primary splice isoforms S97 and A: both of which we found are present at the AIS. These Dlg1 isoforms have multiple binding domains including 3 PDZ binding domains making it a likely candidate for the recruitment and organization of protein complexes at the AIS. We hypothesize that there is a hierarchy of interactions that recruit potential complex members to the AIS, and that specific isoforms of Dlg1 play a role in complex formation. Using isoform specific-RNAi knockdowns and mutant studies, this project will determine which Dlg1 isoforms function at the AIS. We will test how loss of these isoforms alter AIS structures—specifically the localization of Bsg, PMCA, and Shal—and determine the hierarchy of interactions of these complex members. AIS structures are highly conserved throughout both vertebrate and invertebrate neurons. Consequently, this research will help in our global understanding of the AIS in unmyelinated neurons in all animals.

593T **A novel role for the piRNA pathway in synaptogenesis through transposon regulation** Peter G M›Angale, Devyn Oliver, Gimena Alegre, Jasmine Graslie, Alia Ohira, Travis Thomson Neurobiology, University of Massachusetts Chan Medical School

We recently discovered that the endogenous retrovirus, Copia, is a negative regulator of synaptogenesis, at the *Drosophila* neuromuscular junction. While Copia has this physiological role, it is a typical example of a retrotransposon, and its activity can cause genome damage when dysregulated. To define how Copia is regulated at the Drosophila neuromuscular junction (NMJ), we tested the canonical germline transposons repression piRNA pathway. The piRNA is a small RNA pathway that evolved alongside transposable elements to reduce the parasitic nature of these mobile genetic elements to maintain genome integrity. Through an analysis of existing sequencing data there is evidence that piRNAs, albeit not highly expressed, are present in somatic tissues, especially those of a size that associates with Aubergine, one of three piwi-type Argonautes (AGOs) in Drosophila. We use an aub-Gal4 reporter line to show that aub is present at the larval Drosophila NMJ, that a reduction of Aub by RNAi at these NMJs result in a decrease in synaptogenesis, and we observe an increase in TE expression, including Copia. We observe Aub-mutant NMJs in both larvae and adults have measurable defects beyond synapse formation, including locomotor and longevity defects, suggesting the piRNA pathway, or a portion of it is regulating Copia and other TEs to protect somatic tissue from TE damage. Though it is not clear whether this regulation is through the piRNA pathway members with piRNAs, our RNA-seq data show a compendium of differentially expressed genes in both the larval CNS and body wall muscles. Taken together, these data alludes to a potential role fort he piRNA pathway member, aub, in regulating synaptogenesis.

594T **Testing the Role of Discoidin Domain Receptors in Nociception** Victoria Lopez, Stephanie Mauthner, Liping He, W. Dan Tracey Indiana University

Nociception is the sensory process that detects noxious, or potentially tissue-damaging stimuli. Using *Drosophila* larvae as a model, we are investigating the functions of the *smoke alarm (smal)* and *discoidin domain receptor (ddr)* genes. We previously identified the *smal* gene for its role in nociception behavior and nociceptor morphology. Larvae lacking *smal* display hypersensitive nociception behavior and show reduced dendrite branching in their nociceptors. Both *smal* and *ddr* encode homologs for mammalian Discoidin Domain Receptors (DDRs). Mammalian DDRs are receptor tyrosine kinases that are activated by binding collagen. Analysis of *smal* and *ddr* coding sequences in flies suggest that only proteins encoded by the *ddr* locus contain an intracellular kinase.

To determine the relationship between *smal* and *ddr*, we are investigating three alternative hypotheses: (1) DDR signals through Smal via cross phosphorylation, (2) Smal negatively regulates DDR, and (3) Smal and DDR signal independently of each other. In the first hypothesis, we predict that *smal* and *ddr* LOF mutants would have similar nociception phenotypes, and a *smal ddr* double mutant would have phenotypes like either single mutant. In the second hypothesis, we predict that *smal* and *ddr* mutants would have opposite phenotypes. In the third hypothesis, we predict that a *smal ddr* double mutant would display a combinatorial phenotype compared to the single mutants.

Using CRISPR/Cas9 site directed mutagenesis, we have generated a *smal ddr* double mutant. We observed the dendrite morphology of larval nociceptors in this double mutant and in *ddr* single mutants. Based on the nociceptor morphology of these mutants, we hypothesize that *ddr* mutants will phenocopy *smal ddr* double mutants. In addition, RT-PCR data shows that *ddr* is overexpressed in our *smal* mutant, which has reduced dendrite branching in nociceptors. Interestingly, we also found that overexpression of *ddr* in larval nociceptors is sufficient to reduce dendrite branching. Our evidence suggests that *ddr* functions with *smal* to reduce dendrite branching in larval nociceptors. Further analysis of the double mutant compared to *smal* mutants and *ddr* mutants will allow us to understand epistatic relationships and the role of these genes in nociception and neural function.

595T **Evolutionary conservation of midline repulsive signaling by Robo family receptors in flies and mice** Alli Loy, Trent Daiber, Tim Evans University of Arkansas

As the nervous system develops in animal embryos, neuronal axons are guided to their synaptic targets by extracellular cues that signal through axon guidance receptors expressed on the surface of the growth cone. In bilaterian animals, one of the important decisions made by axons in the embryonic nervous system is whether or not to cross the midline. The Roundabout (Robo) family is an evolutionarily conserved group of axon guidance receptors that regulate midline crossing in a wide range of animal groups, by signaling midline repulsion in response to their ligand Slit. Despite their strong evolutionary conservation, it is unknown if the mechanisms of Robo signaling are conserved across different species.

Can Robo receptors from mice regulate axon guidance decisions in Drosophila embryos? To investigate the evolutionary conservation of Robo signaling mechanisms, we express Robo receptors from mice in fly neurons during embryonic development. We find that mammalian Robo receptors can repel axons from the midline in *Drosophila* embryos, and can partially rescue midline repulsion in *robo1* mutants, suggesting that the mechanisms by which they signal midline repulsion may be conserved in insects and mammals. However, mouse Robo receptors cannot signal midline repulsion in fly neurons as effectively as *Drosophila* Robo1, suggesting that there is likely also evolutionary divergence in Slit binding affinity or the specific signaling mechanisms that Robo receptors employ to activate midline repusion in fly vs mouse neurons.

596T **ELAV/Hu family RNA binding proteins regulate neural mRNA processing, cell fate, and differentiation** Binglong Zhang[1], Eric Lai[1], Binglong Zhang[2] [1]Development, MSKCC, [2]MSKCC

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Abstract:
The ELAV/Hu family consists of several RNA-binding proteins (RBPs) that are predominantly expressed in neurons. Indeed, antibodies to the founding member Elav are the most widely-used reagent to label post-mitotic neurons in Drosophila. Although Elav stands for “embryonic lethal, abnormal vision”, *elav* knockouts can survive for several days as arrested 1st instar larvae. However, this is strongly enhanced by knockout of its paralog, Fne («found in neurons»), since the double mutants die within 36 hours of removal from the egg shell. These fail to express ~1000 neural-specific isoforms, including alternatively spliced transcripts and globally extended 3› UTRs. Therefore, ELAV/Hu RBPs are crucial regulators of neural-specific mRNA processing. To begin to elucidate the developmental consequences of these mutants, we conducted a series of cytological studies, as well as bulk and single-cell RNA-sequencing from dissected L1-CNS. As expected for global neural isoform regulators, we observe numerous defects in terminal neural differentiation. However, more surprisingly, the double mutants harbor novel populations of cells with elevated Notch signaling and expression of neural stem cell markers. Therefore, ELAV/Hu RBPs not only promote neural differentiation, but also suppress stem cell-like states. We are currently investigating the regulatory basis of how these factors balance neural lineage progression and differentiation.

597T **Investigating Notch’s roles in chromatin dynamics and inheritance within the developing *Drosophila* central brain** Jason T Palladino[1], Claudia Sesso[1], Yuval Cherki[1], Lucas Elms Kim[1], Xin Chen[1,2] [1]Biology, Johns Hopkins University, [2]Howard Hughes Medical Institute

Proper development depends on asymmetric cell division (ACD), a process by which dividing stem cells produce a renewed stem cell and a differentiating cell. Many intrinsic and extrinsic factors guiding ACD have been found, however, the role of chromatin in ACD is poorly understood. Previously, our lab discovered asymmetries in histone and histone post-translational modification (hPTM) inheritance in *Drosophila* male germline stem cells (mGSCs). Disruption of these asymmetries results in both stem cell loss and overpopulation of progenitor cells phenotypes, suggesting that asymmetric histone inheritance is an essential process in tissue health. Further, deterioration of this process may be common among diseases including tissue degeneration and cancer. Investigation into other adult stem cell lineages showed asymmetric histone inheritance, suggesting it is a general mechanism. However, whether histone inheritance patterns influence cell-fate decisions for more potent developmental stem cells remains to be determined.

To interrogate the role of histone inheritance in development, I use *Drosophila* neural stem cells, neuroblasts (NBs), a well-studied model for ACD. Similar to mGSCs, inheritance of total H3 and H4 in type I NBs (NB-Is) is also asymmetric, suggesting histone density asymmetry is conserved between mGSCs and NB-Is. Despite commonalities, histone inheritance in NB-Is is distinct from mGSCs in several ways, such as the polarity of histone inheritance alternating between dividing NBs. As NB progeny identity is also dynamic, I hypothesize inheritance of histones and associated epigenetic information contributes to cell-fate determination in NB lineages.

Notch plays critical roles in neurogenesis, including NB maintenance and cell fate progression of NB progenies. Recently, Notch has been implicated in distinguishing sister neuron identities by regulating chromatin accessibility. Indeed, overexpression of Notch in NB-Is not only results in more accessible chromatin, but also enhances asymmetric histone inheritance. Further dissection of Notch activities in NB-Is throughout neural development will elucidate the relationship between epigenetic inheritance and complex cell-fate decisions, which I will discuss further.

598T **A new null allele of *wit*, encoding a Type II BMP receptor: Lethal phase, NMJ phenotypes, and comparison to existing alleles** Patrick McGraw, Aidan Rodriguez, Pam Vanderzalm Biology, John Carroll University

The proper connectivity of the nervous system is critical for its function. This involves both correct target acquisition by migrating axons and subsequent growth and maturation of the synapse. The canonical Bone Morphogenic Protein (BMP) pathway is essential for the development of glutamatergic synapses in both vertebrates and invertebrates. In *Drosophila melanogaster,* the neuromuscular junction (NMJ) is used as a model for AMPA-type excitatory synapses of the mammalian central nervous system. Retrograde BMP signaling from postsynaptic muscle to presynaptic neuron is required for the scaling growth of synaptic termini proportional to larval muscle growth. In the absence of any BMP signaling, NMJs severely undergrow and do not release neurotransmitter normally, leading to impaired locomotion.

About twenty years have passed since the identification of the core BMP signaling components regulating synaptic growth and function and the community has long relied on transheterozygous combinations of alleles generated by EMS and generously shared through the Bloomington Drosophila Stock Center. Many labs have used these alleles over the past two decades to elaborate on BMP pathway regulation and NMJ development. However, a thorough description of the molecular nature of the two most commonly used alleles, wit^{A12} and wit^{B11} is lacking. Both are presumptive null alleles: *A12* is described as an early stop codon in the extracellular domain of the protein, however the *B11* allele was never defined molecularly.

We undertook an analysis of the publicly available alleles, examining lethal phase, bouton and active zone number, and sequencing to confirm *A12* and describe *B11*. We simultaneously made a new null allele of *wit* using CRISPR/CAS9, replacing the locus with an attP landing site, and compared the new allele with the *A12* and *B11* alleles for the above phenotypes. Our analysis to date will be presented comparing all three alleles, as well as a deficiency of the region.

Lastly, the only available antibody against Wit does not permit visualization of the endogenous protein at the NMJ; it only appears when *wit* is overexpressed. We used PhiC31 recombination to replace the attP cassette with a wild-type version of *wit* with a C-terminal V5 tag, permitting us to investigate the endogenous localization of Wit at the NMJ with a commercially available epitope-tag antibody. Preliminary results demonstrating Wit localization at the NMJ will be shared.

599T **Deciphering cell-intrinsic versus cell-extrinsic cues guiding neuronal morphology and connectivity** Megan R Radler, Chris Q Doe Institute of Neuroscience, University of Oregon

Neurons are highly polarized cells with complex and diverse morphologies, which are crucial for their connectivity and function. How neurons acquire their morphologies and initiate synaptic connections with sub-cellular specificity is unclear. Within a neuron's environment, there are cell-extrinsic cues that are critical for neurite morphogenesis and development; morphogenic gradients, cell-cell interactions, extracellular matrix, and neuronal activity are crucial for neurite initiation, axonal pathfinding, and synaptic specificity. Meanwhile, the molecular identity of neurons is conferred during neurogenesis, where neural stem cells asymmetrically divide to produce differentiated daughter cells. The resultant neurons inherit a unique suite of transcription factors based on their physical location and temporal birth window, resulting in diverse gene expressions and varied molecular identities. Previous work has shown that alteration of neuronal molecular identity can result in incorrect neuronal morphology and connectivity. How cell-intrinsic and cell-extrinsic cues guide neuronal morphogenesis and synapse formation, and whether and how they are integrated is poorly understood. To address this gap in knowledge, I have established a neuronal culture system using neural stem cells from Drosophila melanogaster embryos, labeled using specific genetic drivers and fluorescent reporters. Using this system, I have begun to probe the morphology and connectivity of the circuit formed by the sensory neuron dbd and the interneuron A08a within the Drosophila larval VNC. Cultured A08a and dbd neurons were analyzed and compared to both annotated skeletons within a Transmission Electron Microscopy L1 larval brain volume, as well as developmental time-matched in situ neurons sparsely labeled with Multi-Color Flip Out technique. I found that when reared in culture, A08a retains several key characteristics of its in vivo morphology, including its distal Y-bend and formation of 2-4 medial dendritic branches. While dbd tends to maintain its small dendritic domain and single long axonal process, most cultured dbd neurons do not maintain the terminal axonal fork that is present in situ. Further experiments and analyses hope to reveal what aspects of neuronal morphology, and eventually connectivity, are determined by cell-intrinsic programming, versus which are decided by cell-extrinsic cues.

600T **Investigating Defective Phagocytosis in Draper Deficient *Drosophila* Brains** Cheng Yang (Jason) Shi, Guangmei Liu, Iqra Amin, Kim McCall Biology, Boston University

Clearance of apoptotic cells via phagocytosis is crucial for maintaining tissue homeostasis, particularly in the central nervous system where glial cells play a pivotal role in removing dead cells. Disruptions in this process can lead to various pathological conditions, including autoimmune and neurodegenerative diseases. The receptor Draper (Drpr) is essential for glia-dependent phagocytosis in *Drosophila*, and its knockdown results in the accumulation of neuronal corpses, actively dying glia, and age-dependent neurodegeneration. The corpses in adult -deficient flies are primarily neurons that die during development. TUNEL staining has shown that the number of corpses remains constant throughout the flies' lifespan, even though the neurodegeneration phenotype indicates that neurons continue to die. This suggests that the corpses that arise during adulthood could be phagocytosed. To uncover cells participating in clearing these corpses, we expressed Green Fluorescent Protein (GFP) in glia using the Gal4/UAS gene expression system and introduced pHRed, a genetically engineered pH sensor, into neurons with the LexAop system. pHRed fluoresces red under acidic conditions, serving as an indicator of engulfment. To visualize engulfment of dying glia, we co-expressed phRed in glia expressing GFP. We have found that *drpr* mutant glia are capable of engulfing dying cells, both glia and neurons. The number of engulfed cells decreases as flies age. Moreover, the size of pHRed+ signals significantly increases in flies with *drpr*-deficiency and increases as flies age, indicating that there are processing defects in -deficient glia and old flies. Future directions will explore candidate proteins that mediate engulfment in the absence of *drpr.*

601T **Function of *frazzled* is conserved in insects.** Piyasi Ghosh[1], Timothy A Evans[2] [1]BISC, University of Arkansas, [2]University of Arkansas

Axon guidance in bilaterians is often controlled by signaling pathways in the nervous system that determine the fate of the axons, for example, if they cross the midline or not. One such pathway involved is the ligand-receptor pair Netrin (Net) and Frazzled (Fra), which is also known as the "attractive pathway". Net and Fra together promote midline crossing of axons in insects and other bilaterians. Orthologs of the pathway receptor Fra in insects is Deleted in colorectal cancer (DCC) in vertebrates, and UNC-40 in *C.elegans* are widely conserved in bilaterians. How well are the regulatory roles of these proteins conserved is not well understood. Hence, this project aims to compare the direct evidence of the midline attractive roles of the Frazzled receptor in the flour beetle (*Tribolium castaneum*) and fruit fly (*Drosophila melanogaster*) using CRISPR/Cas9 gene editing tools. In this project, we rescued the role of Frazzled in promoting the midline crossing of axons in *Drosophila* embryos using an HA-tagged Frazzled from *Tribolium*.

602T **Effects of early-life infection and immune signaling on neuroblast reactivation in *Drosophila* larvae** Omina Nazarzoda[1], Michelle L Bland[2], Sarah E Siegrist[3] [1]Neuroscience Graduate Program, University of Virginia, [2]Pharmacology, University of Virginia, [3]University of Virginia

Building a healthy adult brain is a complex process. Neural stem cell proliferation and differentiation to make neurons and glia, neuropil extension, and synaptogenesis followed by pruning depend on optimal nutrient, resource, environmental and genetic conditions. These processes are tightly regulated because errors can have severe impacts on the developing brain and consequent adult behavior. For example, infection and aberrant immune activation, especially early in development, is associated with disrupted growth and cognitive development in children. The fruit fly *Drosophila melanogaster* has been used extensively to study both neural development and immune signaling. From these studies we know that *Drosophila* neural stem cells, or neuroblasts, grow and proliferate in a nutrient-dependent manner through growth signaling pathways and give rise to the neurons and glia of the brain. *Drosophila* has two humoral immune pathways, Toll and Imd, and data from our labs show that chronic Toll signaling results in reduced larval growth and small adults. We hypothesized that an early-life infection would activate immune signaling in larvae and lead to disruptions to brain development. Indeed, infecting freshly-hatched larvae with *Erwinia carotovora carotovora 15* (*Ecc15*) induces the antimicrobial peptide Drosomycin, a Toll pathway target. Feeding *Ecc15* to freshly-hatched larvae slows their growth and results in smaller adults compared to uninfected controls. In uninfected larvae, neuroblast reactivation from quiescence occurs by ~48 hours after hatching. However, our results show that *Ecc15* infection delayed neuroblast reactivation by 24 hours. Likely as a consequence of this delay, the infected larvae have smaller brains than controls. Activating Toll signaling in the absence of infection also results in smaller larvae and lower neuroblast proliferation rate. These results so far suggest that infection perturbs neural development, and the effects might be mediated by the immune system. Future work will be conducting RNAi experiments to silence components of immune pathways and assess the effects of infection on brain development. Our established larval infection model together with the expansive *Drosophila* genetic toolkit will enable us to unravel the biology of brain development and plasticity in response to early-life infection and immune signaling challenges.

603T **Exploring pH dynamics in stem cell fate regulation** Bernice Lin[1], Isabella Maag[2], Ashley Bielawski[2], Beverly Piggott[2] [1]Neuroscience, University of Montana, [2]Division of Biological Sciences, University of Montana

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

604T **A Conserved RNA Binding Protein Regulates Lipid Storage and Metabolic Pathways** Jordan Goldy, Heath Dunlop, Anita Corbett, Kenneth Moberg Emory University

The brain plays a central role in systemic metabolism by sensing nutrient status and modulating release of circulating peptide hormones which alter the balance between storage vs. breakdown of high-energy fats. This brain-metabolism link is evident in clinical data collected from groups of intellectual disability (ID) patients as elevated risk of metabolic defects, including obesity, elevated triglycerides (TGs), or high blood sugar. One group of heritable IDs are caused by mutations in genes encoding RNA binding proteins (RBPs), which collectively control post-transcriptional processing, translation, and turnover of all RNAs. We have previously shown that loss of the RBP ZC3H14 causes a non-syndromic, autosomal recessive form of ID in humans which can be modeled in *D. melanogaster* by deletion of the ZC3H14 ortholog Nab2. Nab2-deficient flies display a short-term memory defect, altered morphology of neuronal axons and dendrites, and increases in steady-state levels of a small subset of brain mRNAs. Significantly, this group of Nab2-repressed brain RNAs includes *dilp2* and *dilp5*, which encode peptide hormones made by the insulin-producing cells (IPCs), analogs of human pancreatic beta cells, that stimulate lipid storage in fat body (FB) cells. Interestingly, elevation of *dilp2/5* mRNAs only occurs in female brains and, consistent with this sex difference, the FBs in *Nab2* null female larvae contain enlarged lipid droplets while male FBs do not. Neuronal re-expression of Nab2 in otherwise null female larvae rescues lipid accumulation in the FBs, leading to our hypothesis that Nab2 acts within brain neurons to limit levels and potentially translation of *dilp2/dilp5* mRNAs, which in turn control lipid storage in the FB. In support of this model, we have recently collected evidence that Nab2 loss dramatically alters patterns of m^6A methylation on *dilp2/5* mRNAs in the brain, which provides additional support for a role for Nab2 in processing, translation, and/or turnover of these RNAs.

605T **DH31 neuropeptide expression in muscle and motor neurons of *Drosophila melanogaster*: effects on protein levels and mitochondrial metabolism** Marylu M Mardegan de Lima[1], Luciane C Alberici[2], Felipe B Valer[2] [1]Biochemistry, University of Sao Paulo, [2]University of Sao Paulo

The *calcitonin gene-related peptide* (*CGRP*) is a neuropeptide that acts as an agonist for G-protein-coupled receptors, exerting both local and systemic regulatory effects in vertebrates. CGRP influences muscle structure and function via the cAMP/PKA/CREB pathway, regulating protein degradation systems such as autophagy and the ubiquitin-proteasome system. Under pathological conditions, such as muscle atrophy, these systems are upregulated, leading to increased protein breakdown, resulting in muscle mass loss and impaired function of the neuromuscular junction and skeletal muscles. While the effects of *CGRP* on mammalian muscle are well documented, no studies to date have explored the role of CGRP homolog *diuretic hormone 31* (*dh31*) in *Drosophila melanogaster*, in the context of muscle physiology. In this study, we expressed *dh31* in muscles and motor neurons using the UAS-Gal4 system, with *Mef2-Gal4* and *Ok6-Gal4* drivers, respectively. We then investigated phenotypic parameters such as climbing ability, and conducted biochemical and molecular analyses, including protein mass, mitochondrial content (measured by citrate synthase activity), and respiratory function (assessed through high-resolution respirometry). We observed an increased climbing ability in males and female flies expressing *dh31* in muscle tissue, which was associated with enhanced protein levels and improved mitochondrial oxidative capacity. Similar biochemical results were observed when *dh31* was expressed in motor neurons in females, except that climbing ability, which was reduced. Notably, in males, *dh31* expression in motor neurons also increased protein levels but reduced mitochondrial phosphorylation capacity, with no change in climbing ability. Importantly, *dh31* expression did not alter mitochondrial density in any of the groups. Taken together, our results suggest that DH31 positively regulates muscle mass when expressed in both muscle and motor neurons in *D. melanogaster*. DH31 expression in muscles of *Mef2-Gal4>UAS-dh31* males and females also improved climbing performance and mitochondrial function. However, sex-specific differences in climbing ability and mitochondrial function were observed in *Ok6-Gal4>UAS-dh31* animals. These findings highlight the complex, tissue-specific, and sex-dependent effects of *dh31* on muscle physiology in terms of protein levels and mitochondrial capacity.

606T **Distinct Drosophila Mamo Isoforms in Governing Mushroom Body Neuron Identity** TSAI CHI HSU[1,2], Hung-Hsiang Yu[3], Tsai-Chi Hsu[4] [1]Institute of Cellular and Organismic Biology, Academia Sinica, Taiwan, [2]Institute of Cellular and Organismic Biology, Academia Sinica, [3]Academia Sinica, Taiwan, [4]Institute of Cellular and Organismic Biology (ICOB), Academia Sinica, Taiwan

In the Drosophila memory center, the mushroom body (MB), intrinsic neurons (called Kenyon cells/KCs) are sequentially generated into different subtypes (γ, followed by α'β', and finally αβ). As the brain is massively transformed during metamorphosis, the MB also reshapes significantly by different degrees of morphogenesis occurred in pre-existing γ and α'β' neurons and in emerging αβ neurons. Revealing how KC subtypes maintain unique identities and undergo proper morphogenesis is crucial for the comprehension of how the MB is transformed. Our and others previous studies have shown that Maternal gene required for meiosis (Mamo) is critical for the identity establishment and maintenance of α'β' neurons and for remodeling and identity-re-specification of γ neurons. As mamo is a complex gene that encodes four different Mamo isoforms (Mamo C, Mamo D/E, Mamo F/G, and Mamo H/I), we would like to explore whether distinct Mamo isoform functions differently in the development of KC subtypes. Since we have previously shown that Mamo D~G and Mamo H/I isoforms express in different subsets of γ and α'β' neurons, we, here, report that the loss of Mamo H/I isoform causes morphological defect in γ neurons, particularly in the process of γ neuron pruning. In contrast, the loss of Mamo F/G alone and the loss of Mamo D~G isoforms substantially affect the expression of α'β'- and γ-neuron markers, respectively. Beside these distinct phenotypes, intriguingly, an ectopic structure in the MB emerges when the transition of KC subtypes, including from late-born γ neurons to early-born α'β' neurons and from late-born α'β' neurons to early-born αβ neurons, is failed due to distinct Mamo isoform knockdown. Taken together, our results suggest that Mamo isoforms differentially contribute identity specification to generate the proper number of cells with correct morphogenesis in KC subtypes.

607F **Modulation of hyperexcitability in the Na_v-channel mutant *para^{Shu}* by dietary omega-3 fatty acids** Victoria Hand[1], Reid Schuback[1], Aubrey Gray[1], Bailey O'Neal[1], Toshihiro Kitamoto[2], Atulya Iyengar[1] [1]University of Alabama Tuscaloosa, [2]University of Iowa

Voltage-gated sodium (Na_v) channels play a crucial role in the initiation and propagation of action potentials. *Drosophila melanogaster* possesses a single Na_v channel gene, *paralytic* (*para*), which is orthologous to nine Na_v channel genes in vertebrates. In humans, mutations of these genes have been implicated in epilepsy syndromes. In *Drosophila*, the hyperexcitable gain-of-function mutant *para*^{Shu} exhibits seizure-like phenotypes such as convulsions under ether anesthesia, spontaneous spasms (exacerbated at high temperatures), ataxic movements and flight muscle hypercontraction leading to a 'wings-down' posture. Notably, recent reports indicate dietary supplementation of dry milk, milk whey or an omega-3 fatty acid component, α-linolenic acid (ALA) attenuates the abnormal posture and motor phenotypes of *para*^{Shu}. Here, we sought to: 1) establish the concentration ranges for whey or ALA required for the effect, 2) identify specific aspects of motor function modulated by either milk whey or ALA or 3) compare the effects of dietary supplementation in heterozygous *para*^{Shu}/+ females versus hemizygous *para*^{Shu}/Y males. We reared larvae from the respective genotypes on standard fly media (BDSC recipe) supplemented with 0.1 – 2.0% (w/vol) dry milk whey or 0.005 – 0.05% ALA and examined phenotypes in 2 – 5 d. adult flies. In heterozygotes, we found a dose-dependent reduction in the incidence of wings-down posture (< 10 % at the highest concentration vs > 90 % in control flies), while the ether convulsion phenotype was largely unaffected. Notably, the omega-6 fatty acid linoleic acid (0.05%) did not suppress the wings-down phenotype. In male hemizygotes, neither whey nor ALA supplementation affected the wings-down or ether convulsion phenotypes. Using an automated video tracking system, IowaFLI Tracker and an open-field arena, we characterized walking behavior in *para*^{Shu}/+ and *para*^{Shu}/Y flies. Across four measures, total distance traveled, % time active, average speed and path linearity, we found progressive performance improvements in *para*^{Shu}/+ flies following increasing supplementation of milk whey or ALA. In conclusion, our findings highlight the specific applications and limitations of ALA as a suppressant of the *para*^{Shu} phenotype.

608F **A genetic screen of all Drosophila glutamate receptors identifies a new subunit necessary for homeostatic synaptic plasticity** Joshua C Martinez, Chun Chien, Wanying Dong, Nancy L Tran, Allison Chang, Svara Shah, Beril Kiragasi, Dion Dickman Neurobiology, University of Southern California

Synapses must confront perturbations that disrupt transmission throughout development, maturation, and aging. Despite the recent identification of some of the processes involved in synaptic homeostasis, the genes and molecular mechanisms that adaptively increase presynaptic neurotransmitter release remain enigmatic. Recently, our lab identified a presynaptic glutamate receptor (GluR) subunit, KaiRID, and an auxiliary subunit, dSol-1, that function as glutamate autoreceptors. These work to adaptively potentiate presynaptic neurotransmitter release under basal and homeostatically challenged conditions. To determine whether other GluR subunits contribute to these processes, we have systematically mutated, mapped expression, and characterized synaptic function of all 16 GluRs encoded in the Drosophila genome. Each GluR subtype is represented in the fly genome, including Kainate-, AMPA-, NMDA-, Glutamate-gated Chloride, and metabotropic-type GluRs. First, we found that each of the five GluR classes are expressed at the larval neuromuscular junction (NMJ): 9/16 are expressed in motor neurons, 5/15 are exclusively expressed in the postsynaptic muscle, while the remaining 2 GluRs are apparently not expressed in larval stages. We also mapped expression in the adult brain and found all are expressed in various regions except for the 5 muscle GluRs. Next, we generated null mutations in all 16 GluRs using CRISPR/Cas9 gene editing and screened the 9 motor neuron GluRs for potential roles in NMJ growth and function. Surprisingly, each of these GluR mutants are viable and show no major defects in synaptic growth or baseline transmission. Finally, we have identified one additional GluR subunit, the *eye-enriched kainate receptor* (Ekar), to be selectively required for the chronic expression of presynaptic homeostatic potentiation. Ongoing work is now focused on determining whether Ekar associates with KaiRID and dSol-1, and how Ekar selectively promotes chronic expression of homeostatic plasticity.

609F **Steroid hormone-dependent glial-neuronal interaction promotes brain development during *Drosophila* metamorphosis** Eisuke Imura[1], Naoki Okamoto[1,2], Naoki Yamanaka[1] [1]Department of Entomology, University of California, Riverside, [2]Life Science Center for Survival Dynamics, University of Tsukuba

Steroid hormones regulate various aspects of brain development in metazoans. In the fruit fly *Drosophila melanogaster*, the primary steroid hormone ecdysone enters the central nervous system (CNS) via Ecdysone Importer (EcI) expressed in the blood-brain barrier (BBB) and controls brain development during metamorphosis. However, our understanding of the specific cell types that require ecdysone during CNS transformation remains limited. Here, we report that ecdysone acts on glial cells and promotes brain development during *Drosophila* metamorphosis. Blocking ecdysone signaling in entire neurons did not lead to major defects in brain development during metamorphosis, whereas disrupting ecdysone signaling in glial cells severely impaired CNS transformation and showed developmental lethality at the prepupal stage. We further focused on mushroom body (MB) neurons, whose remodeling is well known to require ecdysone receptor (EcR) in a cell-autonomous manner. Although knockdown of *EcR* in MB neurons showed remodeling defects as reported previously, knockdown of *EcI* in the MB did not induce any discernible deficiency in neuronal remodeling, suggesting a ligand-independent function of *EcR* in the MB. Consistent with this, the neuronal remodeling defects induced by ecdysone deprivation in the CNS were rescued by glial cell-specific overexpression of a transforming growth factor-β (TGF-β) ligand *myoglianin* (*myo*) and an engulfment receptor *draper* (*drpr*), both of which are upregulated in glial cells in an ecdysone-dependent manner. In conclusion, our findings suggest that ecdysone is required in glial cells for CNS transformation during metamorphosis, elucidating a steroid hormone-dependent glial-neuronal interaction that drives brain development.

610F **The TRIM-NHL RNA-binding protein MEI-P26 modulates the size of Drosophila Type I neuroblast lineages** Yichao Hu[1], Xiaohang Yang[2], Howard Lipshitz[1] [1]University of Toronto, [2]Zhejiang University

The Drosophila TRIM-NHL RNA-binding protein (RBP), MEI-P26, has previously been shown to suppress tumor formation in the germline. Here we show that, in the Drosophila larval central brain, cell-type specific expression of MEI-P26 plays a vital role in regulating neural development. In early Type I lineages, MEI-P26 is expressed in a complementary expression pattern to another TRIM-NHL RBP, Brain tumor (BRAT), a well-known tumor suppressor in Drosophila neurodevelopment. Knock down of MEI-P26 leads to re-expression of the stem cell marker, DPN, and over-production of neurons. In contrast, ectopically expressed MEI-P26 reduces lineage size by repressing division of GMCs and, thus, neuron production. Prospero (PROS) is a transcription factor known to repress cell cycle related genes. We have shown that MEI-P26 positively regulates PROS expression in Type I lineages; that ectopic expression of PROS phenocopies ectopic expression of MEI-P26; and that, in both cases, Cyclin B (CYCB) expression is downregulated. Importantly, knockdown of PROS in the context of ectopic MEI-P26 rescues the neural lineage. To identify direct targets of MEI-P26 in neuroblast lineages we modified the TRIBE method to allow conditional expression in neuroblast lineages of a CRISPRed endogenous MEI-P26-ADAR catalytic domain (cd) fusion protein. MEI-P26-ADARcd catalyzes A-to-I(G) base editing of MEI-P26-bound RNAs, which can be identified by next-generation sequencing. Using this method, we identified PROS as a direct target of MEI-P26 and further validated this result by MEI-P26 RIP-Seq from larval brains. We hypothesize that MEI-P26 functions to modulate the size of Type I lineages by binding to and promoting the translation of the *pros* mRNA in neurons; PROS, in turn, represses cell cycle genes, thus preventing over-proliferation of neurons. The 'switch-hyperTRIBE' method is generally useful to identify cell-type specific targets of RBPs by ensuring that the RBP-ADARcd fusion protein is expressed in specific lineages at endogenous levels.

611F **Role of *Deformed* (*Dfd*) and other ANT-C *Hox* genes in CNS metamorphosis: Axonal fasciculation and cell migration in cervical connective formation** Linda L Restifo, Brianna M Zahorecz Neurology, University of Arizona College of Medicine

Like other ANT-C *Hox* genes, *Dfd* controls anterior-posterior segment identity by regulating transcriptional targets throughout development. We previously used classical genetics and histology to show that *Dfd* is required for dramatic changes during CNS metamorphosis: separation of the subesophageal zone (SEZ) from the thoracic ganglia (TG) and, in parallel with this morphogenetic movement, elongation of a four-fascicle axon tract to form the cervical connective (CCt) in the neck (PMID:8150208). In the current hypothesis-generating study, we returned to that archival collection of aldehyde-fixed, paraffin-embedded serial sections to find candidate cellular and molecular mechanisms. We focused on the CCt of pharate adults cut in the sagittal plane, viewed at 200X and 400X. *Dfd*-mutant chromosomes used were: *rV8* (*Dfd*^{13}, semi-viable), *rC11* (*Dfd*^{3}, temperature-sensitive lethal), *rW21* (*Dfd*^{16}, null), and *Df(3R)Scr* (deletes ANT-C: 84A1,2–84B1,2).

We observed varying degrees of axon-bundle-fusion failure within the CCt, especially between ipsilateral dorsal and ventral fascicles. Penetrance, which generally paralleled severity, was as follows: Dfd^{3}/Dfd^{3} at permissive temperature (30%) < Dfd^{13}/Dfd^{16} (36%) < Dfd^{3}/Dfd^{3} at restrictive temperature post-embryogenesis (41%) << $Dfd^{13}/Df(3R)Scr$ (93%). In contrast, 11% of mixed genetic controls showed mild fusion failure. Qualitatively, mutant CCt bundles often lacked the smooth contours and straight trajectories of control CCt. There were also abnormalities in size or position of the axon bundles that contribute to the CCt in the posterior head and anterior thorax. Our prime suspects for these CCt phenotypes are abnormal expression levels or timing of Fasciclin adhesion proteins.

In most mutants with severe CCt fusion failure, a column of densely packed ectopic cells resembling neurons was seen between the axon bundles. In some specimens, both fusion failure and ectopic cells were patchy in distribution. Aberrant Fasciclin expression could also disrupt cell migration, leading to ectopic cells in the CCt. Fusion failure and ectopic cells between bundles were not fully correlated in individual specimens, as substantial gaps between unfused CCt axon bundles could be devoid of ectopic cells. Compared with axon-bundle-fusion failure, penetrance of ectopic cells was lower, especially in the simple *Dfd* mutants: Dfd^{3}/Dfd^{3} at permissive temperature (0%) < Dfd^{3}/Dfd^{3} at restrictive temperature post-embryogenesis (12.5%) < Dfd^{13}/Dfd^{16} (18%) << $Dfd^{13}/Df(3R)Scr$ (86.7%). Notably, with the hypomorphic allele Dfd^{13}, penetrance of both phenotypes was 2.5- to 5-fold higher over the large deletion compared to over the null. This implicates haploinsufficiency of other ANT-C genes as contributors to abnormal CCt morphology. Our histology-based interpretations and hypotheses are readily testable.

612F **Conserved progenitor transcription factors are required for the proper development of dorsal and ventral fan shaped body neurons.** Michael Velasquez Department of Biology, University of New Mexico

Mike Velasquez, Mubarak Hussain Syed

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Olfactory navigation and sleep are complex behaviors- mediated by an evolutionarily conserved insect brain region, the central complex. Recent connectome studies of the central complex have described about 3000 neurons of about 250 different types. What genetic and developmental programs regulate the formation of the CX cell types is poorly understood. Previous work has identified that ventral fan-shaped body (vFB) neurons, which are odor-encoding neurons, and dorsally projecting fan-shaped body (dFB) neurons, which are sleep-regulating neurons, are generated from the same neural stem cell (NSC), the Dorsolateral 1 (DL1) Type 2 NSC. Generation of distinct neural types requires the combination of temporal transcription factor (TTF) cascades in both Type 2 NSCs and Intermediate Neural Progenitors (INPs), which are generated by asymmetric divisions of the DL1 NSC. We want to show how INP TTFs regulate these complex behaviors by regulating the differentiation and specification of dFB and vFB neurons. Our preliminary work has demonstrated that conserved genes; Scarecrow, Eyeless, and TFAP2 are involved in the proper development of vFBs. Knockdown of these TTFs in INPs leads to a loss of vFB neurons, suggesting they might play an important role in their specification. Currently, we are performing overexpression of these TTFs in INPs, and assaying the fate of vFBs and dFBs is necessary to elucidate their sufficiency in determining the fate of these neuron types.

613F **Activity dependent development of larval mechanosensory neurons** Nova Qi[1,2], Toke Ibraheem[1,2], Sergio B Garcia[1,2], Wesley B Grueber[1,2] [1]Columbia University, [2]Zuckerman Institute

Neuronal circuit formation relies on a precise integration of growth, guidance cues, and neuronal activity for neurons to properly reach targets and connect with downstream partners. While spontaneous neuronal activity has been shown to play a prominent role in fine-tuning axon terminal projections in mammals, the role of activity in establishing Drosophila neural circuits has been less well studied. Recent studies have detected spontaneous activity during development of a group of Drosophila mechanosensory neurons, the chordotonal (ch) neurons. These studies also showed that activity during embryonic development is required for proper larval crawling behavior. We used single cell labeling coupled with machine learning analysis of axon morphology to investigate whether functional defects caused by dysregulated activity may be explained by changes in ch axon wiring. We found that ch neurons normally adopt highly complex axon branching and dense filopodial extensions in early developmental stages, with terminal size increasing throughout development. The axons of silenced ch neurons were consistently smaller compared to age-matched non silenced controls. Silencing in larval stages led to morphologies in mature animals that resembled those observed in immature stages, features which have been observed in larval motor and olfactory neurons and zebrafish retinal ganglion cells. Conversely, when ch neurons were constitutively activated, we found that mature axon terminals were less branched, showed fewer filopodia, and mistargeted into regions that were not normally occupied by ch axons. Our approach thus provides a system to study mechanisms of activity regulated development in vivo while leveraging the high neuron accessibility and readily available genetic tools in Drosophila. We will present our initial investigations into the molecular and cellular basis of activity-regulated neuronal morphogenesis of ch neurons. Our results point to new roles for neuronal activity in permitting neuronal maturation and axon refinement in Drosophila, potentially paralleling the well-known roles in mammalian circuits.

614F **Heterochromatic silencing of immune-related genes in glia is required for BBB integrity and normal lifespan in *Drosophila*** Shunpan Shu, Mingsheng Jiang, Xue Deng, Yanshan Fang IRCBC, SIOC, Chinese Academy of Sciences

Glia and neurons face different challenges in aging and may engage different mechanisms to maintain their morphology and functionality. Here, we report that the adult-onset downregulation of a *Drosophila* gene *CG32529/GLAD* leads to shortened lifespan and age-dependent brain degeneration, which exhibits cell type and subtype-specificity, involving mainly surface glia (comprising the BBB) and cortex glia (wrapping neuronal soma). Glial knockdown of *GLAD* disrupts glial and BBB integrity. The expression of *GLAD* mRNA in fly heads is decreased with age and RNA-seq reveals a profound upregulation of immune-related genes. We further experimentally demonstrated that this immune over-activation is the cause, but not merely a consequence, of the degenerative phenotypes. Furthermore, we uncover that *GLAD* encodes a heterochromatin-associating protein that binds to the promoters of an array of immune-related genes, keeping them silenced during the cycle of cell division. Together, our findings demonstrate an essential role of heterochromatic gene silencing of immune-related genes in fly glia, which is required for the BBB and brain integrity as well as normal lifespan.

615F **Investigating how acetylation of lysine 394 impacts axon morphogenesis and microtubule behavior in *Drosophila melanogaster*** Chloe J Welch[1,2], Sophia Trujillo[1], Jill Wildonger[1,2,3] [1]School of Biological Sciences, Cell & Developmental Biology, University of California, San Diego, La Jolla, CA, [2]Biological Sciences Graduate Program, School of Biological Sciences, University of California, San Diego, La Jolla, CA, [3]School of Medicine, Pediatrics, University of California, San Diego, La Jolla, CA

Disruptions in the microtubule cytoskeleton result in various neurodevelopmental and degenerative diseases, which currently afflict a large number of the human population. Microtubule function in neurons is regulated by post-translational modifications—including acetylation—and one consistently identified acetylation site in mammals and *Drosophila melanogaster* is alpha-tubulin lysine 394 (K394). Previous work in flies demonstrated that the acetylation-blocking mutation K394R results in decreased microtubule stability in axon terminals at the larval neuromuscular junction. Our current project aims to investigate how K394 acetylation influences axon morphogenesis and microtubule behavior *in vivo* by capitalizing on an adult brain model. During development, growing axons must precisely establish connections with their appropriate target cells in order for the neural circuitry that governs processes like thought and behavior to function properly. Defects in axon outgrowth are common to a number of neurological disorders, including autism spectrum disorder. The *Drosophila* mushroom body, a well-established model of axon patterning in the adult brain, is a midbrain structure formed by a pair of neuropils that include axon trajectories in medially- and dorsally-projecting lobes termed alpha, beta, and gamma. The mushroom body is comparable to the vertebrate hippocampus and, similarly, is critical for higher order sensory integration, learning, and memory. Generally, axons in the medially-projecting beta lobes terminate near the midline of the brain but do not cross it. We have found that K394R mutants, in which acetylation of K394 is disrupted, exhibit a midline crossing phenotype. We have also found that overexpression of histone deacetylase 6 (HDAC6)—which deacetylates K394R—results in a midline crossing phenotype, indicating a potential link to acetylation and mushroom body development. Furthermore, we found that exposure to the microtubule-destabilizing reagent nocodazole exacerbates this phenotype in a dose-dependent manner which suggests that decreased microtubule stability may contribute to this phenotype. By evaluating a cellular phenotype common to neurodevelopmental disorders, our research supports the use of K394R as a foundational starting point towards potential directions for building a model of neurological disease.

616F **Peptidergic Neuron Function Requires Balanced Lipid Remodeling** Adriana Bibo[1], John Vaughen[2] [1]Anatomy, University of California San Francisco, [2]University of California San Francisco

Lipids play critical functions in all cells and are particularly important for the brain, a lipid-rich organ composed of morphologically complex neurons that communicate by rapid vesicle fusion and neurotransmitter release. Across species, developing brains robustly accumulate polyunsaturated fatty acids (PUFAs), which contain multiple carbon-carbon double bonds in hydrophobic tails. These PUFA-bearing lipids profoundly alter membrane flexibility, vesicular fusion, and lipid-protein interactions. Critically, depleting the essential fatty acids required to build PUFA phospholipids impairs neural function from flies to human, yet how PUFA lipids function in brain cells remains poorly understood. Using liquid chromatography-mass spectrometry (LC-MS), our lab found that specific PUFAs increase dramatically in late brain development in *Drosophila*, coinciding with the onset of electrical activity. Moreover, these PUFA species were not detected in food or other tissues like adipocytes. Because the fly does not eat during pupal development, a genetic program must govern PUFA accretion in the brain.

One enzyme family responsible for lipid remodeling is Membrane-bound O-acyltransferases (MBOATs), which incorporate PUFA acyl chains into phospholipids. *Drosophila* MBOATs have not been studied in the brain but are expressed in neurons and glia over development. We first tried to remove and overexpress MBOATs in neurons, glia, or both. Strikingly, we found that overexpressing the MBOAT *Nessy* (*nes*) in mature neurons caused protein aggregates and wing expansion defects. We traced these deficits to a population of peptidergic neurons (CCAP+), which produce the neuropeptide, bursicon, responsible for wing expansion. Interestingly, upregulation or downregulation of electrical activity in CCAP+ neurons caused a similar wing phenotype. We are now investigating if overproduction of Nes and Nes-modified lipids hyperactivates peptidergic neurons to impair wing expansion and how Nes overproduction affects lipid balance in secretory pathway vesicles. This project will begin to unveil the mystery of how PUFAs and lipid remodeling enzymes function in neurodevelopment and neuron communication.

617F **Linking neuronal identity specification to differentiation using single-cell multiomics** Rose Coyne[1], Krish Pandey[1], Cathleen Lake[1], Yen-Chung Chen[2], Claude Desplan[2], Mehmet Neset Ozel[1] [1]Stowers Institute, [2]New York University

During neurogenesis, signaling molecules and transcription factors (TFs) pattern neural progenitors across space and time to generate the numerous cell types that constitute neural circuits. In postmitotic neurons, these identities are established and maintained by another class of TFs known as terminal selectors. We and others have described in the *Drosophila* optic lobe both the patterning factors in its progenitors and the unique combinations of terminal selectors that encode the identity of its >200 neuronal types. Very little is known about how these two regulatory programs interface within newborn neurons.

To understand how selector combinations are determined, we performed simultaneous scRNA and ATAC-seq (multiomics) experiments on the optic lobe during neurogenesis. We reconstructed complete trajectories from neuroepithelia to differentiated neurons, integrating both gene expression and chromatin accessibility. We investigated the regulatory links between temporal TFs in neuroblasts (that are generally not maintained in neurons) and the terminal selectors in the unicolumnar neurons of the medulla neuropil, which largely disregard spatial patterning to be produced from all domains.

Our results indicate that final terminal selector combinations are determined by a complex and dynamic gene regulatory network that unfolds during the first few hours of a neuron's life after its terminal division. Temporal TFs inherited from neuroblasts activate a preliminary set of selectors through 'initiation' enhancers, which often also integrate the Notch status of sister neurons. A distinct set of 'maintenance' enhancers stabilize the final selector code by integrating cross-(and self-)regulatory interactions among these initial TFs. In a striking example, Vsx1/2 (known spatial factor of a specific domain) are expressed as terminal selectors in Dm2 neurons regardless of spatial origin. We identified the enhancers that control Vsx1/2 expression in Dm2, which are distinct from their spatially patterned enhancers and appear to be regulated by temporal TFs instead. In the closely related Mi15 neurons that do not express Vsx1/2, a transiently expressed TF, Pdm3, is required to suppress Dm2 fate. Therefore, neuronal identity specification is a multi-step regulatory program that resolves the developmental history of each neuron into a unique and stable terminal selector code, wherein the same TFs can enact distinct regulatory codes at different steps and across cell types.

618F **Patterns of brain Ferritin expression in the Drosophila divalent cation transporter mutant *Malvolio*** Breanna Leach[1], Prabriti Neupane[2], Mary Short[2], Jai Scarboro[3], Rajprasad Loganathan[2] [1]Biomedical Engineering, Wichita State University, [2]Biological Sciences, Wichita State University, [3]Biological Scienes, Wichita State University

Malvolio (Mvl) is the Drosophila ortholog of the mammalian Solute Carrier Protein Slc11a2, which transports divalent metals, including iron. The function of Mvl in the developing Drosophila brain is unclear and the developmental anomalies of the brain in *Mvl* mutant, if any, have not been investigated. Our objective was to determine potential physiological defects, if any, in the brain of *Mvl* mutants. We tested iron availability in the brain of *Mvl* loss-of-function mutant, Mvl^{exc1}. We used the Ferritin 1 HCH GFP protein trap fly line as the control. Brain tissue from both the control and mutant animals were dissected and the Ferritin GFP levels at both the larval and adult stages were recorded. Ferritin 1 GFP intensity was used as the marker of iron availability for comparison between the mutants and controls. We confirmed that the loss of Mvl results in lack of iron storage in the midgut iron cells. Contrary to our expectation, we observed differential and sharply contrasting regions of Ferritin expression in the *Mvl* mutant brains compared to controls. The optic lobes expressed high levels of Ferritin (high GFP) in the *Mvl* mutants compared to the central brain lobe in a pattern that persisted during both larval and adult stages. We are currently investigating whether the high Ferritin level in the mutant brain is a result of increased Ferritin expression in the neuron, Glia, or both. We are also investigating the significance of regions marked by contrasting levels of signal in the mutant Vs. control brains. The finding that *Mvl* mutant brain tissue (optic lobe) has higher Ferritin expression compared to the control suggests one or more of the following scenarios: (i) Despite the loss of Mvl, brain tissue can access iron, via non-Mvl dependent cellular uptake of iron, and/or (ii) Ferritin expression in brain tissue is uncoupled from cellular iron availability. We are testing the latter hypothesis by implementing dietary iron restriction during Drosophila development.

619F **USP8 and Prosap Manipulations Lead to Formation of Ectopic Synapses at the Neuromuscular Junctions of Drosophila Larvae** Shreya Singh, Munachiso Nkeonye-Mbaekwe, Claudia Gualtieri, Fernando Vonhoff Biology, University of Maryland Baltimore County

Autism spectrum disorder (ASD) comprises a variety of neurodevelopmental conditions characterized by challenges in social interactions, communication, and atypical behaviors, with symptoms typically emerging in early childhood. Affecting approximately 1 in 100 children worldwide, ASD presents a broad spectrum of abilities and needs, underscoring the importance of further research into the genetic and environmental factors contributing to autism's onset. Synaptic elimination, or synaptic pruning, is the process during neuronal development by which ectopic synapses on off-target partners -incorrect synaptic connections- are ultimately removed. This process plays a crucial role in shaping the synaptic circuitry in the nervous system. Disruptions to this process have been associated with neurodevelopmental disorders, including ASD. Over 100 candidate genes are implicated in the development of ASD. In this project, I examined the effects of USP8 and Prosap genetic manipulations on the neuromuscular junctions in Drosophila larvae. These genes provide insights into both protein degradation and regulation of synapse formation. I conducted filet dissections on Drosophila larvae to examine synaptic elimination following manipulations of candidate autism-related genes. This procedure involved exposing the musculature, thereby preserving the peripheral nervous connections for further analyses. Following fixation, I conducted immunohistochemistry to visualize and quantify ectopic synapses using a confocal microscope. This project enhances our understanding of how these candidate genes may disrupt the process of synaptic elimination and may provide insight into anatomical and molecular processes underlying ASD pathology.

620F **Assay to measure neuronal responses to mechanical stimuli in intact larvae** Annie X Wang, Jill Wildonger University of California San Diego

Sensory neurons relay information that they receive through their dendrites to the central nervous system, where the information is processed and integrated to generate a behavioral response. The distribution of ion channels within dendrites allows neurons to detect external stimuli. Our lab is investigating the functional significance of variations in the density of the ion channel Pickpocket in the dendrites of class IV dendritic arborization neurons. Pickpocket has been implicated in mediating responses to mechanical stimuli, in particular harsh mechanical stimuli. We observe that Pickpocket channel density is lower in proximal dendrites close to the cell body compared to distal dendrites away from the cell body. Here, we describe the development of a protocol to analyze the functional implications of this ion channel distribution to responses to mechanical stimuli in intact larvae. Our protocol uses GCaMP as a fluorescent reporter of neuronal activity. We describe an approach to monitor changes in GCaMP fluorescence in dendrites before and after poking an intact larva with a Von Frey fiber (Von Frey fibers exert a calibrated mechanical pressure on the animal). This assay enables researchers to monitor neuronal responses to mechanical stimuli without dissecting or otherwise overtly damaging the animal, giving researchers insight into how neurons normally perceive external stimuli.

621F **The role of endocytosis in Pickpocket ion channel distribution in Drosophila sensory neuron dendrites** Savannah Arabe[1], Sanne Pikaar[2], Annie Wang[1], Jill Wildonger[2] [1]School of Biological Sciences, Cell & Developmental Biology, University of California San Diego, [2]School of Medicine, Pediatrics, University of California San Diego

Ion channel distribution in dendrites is important for neuronal function. We recently discovered that the Pickpocket ion channel is differentially distributed in the dendrites of peripheral sensory neurons in developing larvae. Pickpocket channel density is low in proximal dendrites but high in distal dendrites. This change in ion channel distribution correlates with glia-wrapping of the proximal dendrites. Ion channel density is often regulated by endocytic pathways. To determine whether endocytosis plays a role in the differential distribution of Pickpocket channels in dendrites, we used fluorescently tagged endogenous Pickpocket and manipulated endocytosis by disrupting dynamin, known as Shibire in Drosophila, which is a central regulator of endocytosis. Our results reveal that Pickpocket channels undergo dynamin-dependent endocytosis and that the endocytosis of Pickpocket channels is elevated in proximal dendrites. Our data suggest that endocytosis plays an integral role in regulating ion channel distribution along the proximal-distal axis in sensory dendrites.

622F ***Bx42* cooperates with *Prospero* to grow the developing brain** Nicole A Losurdo, Adriana Bibo, Nichole Link Neurobiology, University of Utah

Microcephaly is a rare neurodevelopmental disorder characterized by a reduced orbito-frontal circumference of two standard deviations or more below the average for a child's age group. A majority of microcephaly cases with a known cause are due to genetic mutations in neurodevelopmental genes, but many cases remain undiagnosed. Through a patient-informed loss-of-function screen in *Drosophila*, we identified *Bx42* to be necessary for normal brain growth in the fly. Knockdown of *Bx42* in neural stem cells results in significantly smaller brain lobes, fewer central brain neural stem cells, and a complete absence of mitotic central brain neural stem cells. Further characterization of the remaining neural stem cells shows aberrant nuclear expression of Prospero, indicating that these cells may be quiescent. Furthermore, a double knockdown of *Prospero* and *Bx42* fully rescues brain size, indicating a potential genetic interaction. Not only have we attributed *Bx42* to be a necessary neurodevelopmental gene in the fly, but co-expressing the human ortholog in the *Bx42* knockdown animals rescues the developmental phenotypes, showing its relevance to human disease. *Bx42* is a spliceosomal component and a transcriptional regulator known to modulate many critical developmental pathways in various organs. Our continuing work will look into which pathways *Bx42* controls and help elucidate its role as a potential master developmental regulatory gene.

623F **Investigating the molecular mechanism of wrapping glia development by the transmembrane proteins Kon-tiki and Integrin in Drosophila peripheral nerves** Zhiheng Luo Zoology, University of British Columbia

Glial cells comprise a substantial portion of cells in the nervous system, and their functions are well-conserved across the animal kingdom. One extensively studied function of glial cells is the ensheathment of large-diameter axons by myelinating Schwann cells, which form myelin sheaths. Interestingly, most of the peripheral nerves in humans consist of small-diameter axons ensheathed by another class of Schwann cells known as the non-myelinating Schwann cells in non-myelinated (Remak) bundles. Loss of non-myelinating Schwann cells leads to degeneration of sensory neurons such as the unmyelinated nociceptive C-fibers, resulting in severe neuropathic pain. Non-myelinating Schwann cells play a key role in human peripheral neuropathies associated with conditions like diabetes and aging, which cause the degeneration of small-diameter non-myelinated axons. However, the mechanisms underlying the development of non-myelinating Schwann cells remain poorly understood, and their contribution to peripheral neuropathies is often underappreciated.

In *Drosophila melanogaster,* the wrapping glia serves as the morphological equivalent of non-myelinating Schwann cells in vertebrates. We found that knockdown of the beta-subunit of integrin (βPS, *myospheroid*), results in the loss of axon ensheathment by the wrapping glia, but the binding partners of Integrin in the wrapping glia remain unclear. Integrins can interact with proteins containing Laminin globular (LamG) domains and we carried out a screen to knock down LamG domain-containing proteins specifically in the wrapping glia. Through our RNAi screen, we identified Kon-tiki (Kon). Kon is a transmembrane protein containing two extracellular LamG domains expressed in the *Drosophila* peripheral glia. RNAi-mediated knockdown of Kon mimics the Integrin knockdown phenotype, in which the wrapping glial layers fail to wrap around the axons. Immunostaining revealed that Kon colocalizes with the Integrin beta-subunit in both the wrapping and outer glial layers. We are currently investigating the genetic interactions between Kon and Integrin in promoting wrapping glia ensheathment of axons. Given the conservation of integrins and Kon-tiki, results from this work will shed light on the protein interaction pathways involving the development of non-myelinating glia in all animals.

624F **Investigate the underlying mechanism of dendrite degeneration of adult *Drosophila* peripheral sensory neurons during aging** Shiang-Chi Lin, Min-Hsien Wang, Han-Hsuan Liu Center for Neuropsychiatric Research, National Health Research Institutes

The skin serves as the body's primary defense, essential for sensing and responding to potentially harmful stimuli, such as heat and sharp objects. A decrease in sensory function may increase the risk of injuries, burns, and other hazards. However, the effects of aging on skin sensation are still largely unknown. To explore this, we use the *Drosophila* sensory system as a model to assess age-related changes in skin sensation. Our results indicate that nociceptive neurons in *Drosophila* exhibit degenerative changes similar to those associated with aging in physiological conditions. Specifically, we observed structural degeneration in class 4 dendritic arborization (c4da) sensory neurons, including beaded dendrites and a decrease in dendrite length and tip numbers. Based on a recently published aging fly single nucleus RNA sequencing (snRNA-seq) dataset by Lu *et al*., which reveals a decline in Guanylyl cyclase at 88E (Gyc88E) expression with age, we hypothesized that overexpressing Gyc88E might help slow neurodegeneration. Interestingly, our findings showed that Gyc88E overexpression increased dendrite length in young flies (7 days old) and reduced dendrite tip numbers in older flies (49 days old), suggesting that Gyc88E's effects vary by age. Overall, our findings indicate that peripheral sensory neurons in flies show signs of degenerative phenotypes with aging, and Gyc88E may play a role in regulating dendrite structure differently at various stages of aging.

625S **The role of Lasp in muscle development, structure, and function.** Hayden Dalton[1], Collin Clark[1], Kiel Ormerod[2] [1]Biology, Middle Tennessee State University, [2]Biology, MTSU

Skeletal muscle enables animals to produce movement, facilitating a robust set of behaviors and interactions with their environment. The ability of skeletal muscles to contract is derived from the unique genes and proteins that are expressed within muscles, most notably thick and thin filaments, and elastic proteins. Within *in vivo* systems investigations of these proteins are particularly difficult as they often lead to gross phenotypic changes, compensatory mechanisms, or lethality. To circumvent this limitation, *Drosophila* biologists exploit the Gal4/UAS system to selectively express genetic manipulations in a specific subset of cells or even individual cells. We recently took advantage of these tools to selectively manipulate the expression of sallimus, the ortholog of titin in *Drosophila*, in a single muscle fiber, and showed profound impacts on neuromuscular junction development (NMJ) and muscle structural assembly. Here we build upon these findings to explore the role of another poorly understood structural sarcomeric protein, lasp. The Lasp gene encodes the only member of the nebulin family in *Drosophila*, a family of actin-binding proteins, which are known to regulate sarcomeric I-band length in other systems. Using RNAi to selectively knock-down the expression of lasp in a single abdominal muscle fiber did not impact the viability or morphology of eggs, or any of the instar- stages during development. Using immunohistochemistry and fluorescence microscopy, we quantified changes in muscle structure at the gross and ultrastructural level. Next, we explored the impact these muscle manipulations had on NMJ formation, as well as synaptic communication at the NMJ via electrophysiological recordings. Subsequently, we explored the impacts of muscle-specific manipulation of lasp on muscle force production, and animal behavior using a larval crawling assay. These results further our comprehension of the role of sarcomeric muscle proteins in regulating muscle development, architecture, but further our understanding by characterizing how manipulations in these proteins alter muscle performance and rhythmic locomotory behavior.

626S **Social Experience Induced Modulation of the Blood-Brain Barrier** Shayna N Scott[1], Emily Wu[2], Pelin Volkan[1] [1]Biology, Duke University, [2]Duke University

Social isolation was shown to increase the incidence of neuropsychiatric and neurodegenerative diseases. Emerging new data suggests defective neuroinflammation is an underlying cause of many diseases of the nervous system. The blood-brain barrier (BBB) is a vital immunological component of the nervous system, serving as a selective permeability barrier that protects the brain. Disruptions in the BBB have been linked to dysregulation, edema, and neuroinflammation, contributing to neuronal dysfunction, increased intracranial pressure, and neurodegeneration. Prior research has extensively documented factors influencing the BBB, including diet, aging, the gut microbiome, circadian rhythms, and environmental conditions. Emerging studies have highlighted the impact of psychological and social stressors on the BBB's integrity. This study used *Drosophila* melanogaster and dextran dye injection assays to demonstrate that social isolation increases BBB permeability in Canton S flies. We also examined the knockout (KO) of two distinct pheromone receptors, *Or47b ,* and *Or67d*, finding that these mutations influence BBB leakiness in opposing ways. Additionally, our work indicates that grouping size modulates BBB permeability. Analysis of whole brain transcriptome also demonstrated that multiple regulators of BBB are regulated by social experience, like immune genes, stress response proteins, and circadian regulators. *Or47b* and *Or67d* pheromone circuits contribute to this response in opposing ways. Our findings suggest that social experiences and pheromone signaling pathways induce structural modifications to the BBB, potentially resulting in downstream physiological effects.

627S **Axon Guidance Defects in Robo2 Ig Domain Variants in the *Drosophila* CNS** Elizabeth A Magdich Biol, University of Arkansas

This study investigates the role of Robo2, a member of the Roundabout (Robo) family, in axon guidance within the Drosophila central nervous system (CNS). Robo receptors, initially identified through mutation screens, are known to direct axon pathways by responding to Slit ligands at the CNS midline. Using CRISPR/Cas9 gene replacement, we created variants of *robo2* in which individual proteins domains are deleted. Robo2 variants display unique phenotypes: while certain variants, including $Robo2^{robo2\Delta Ig1}$, $Robo2^{robo2\Delta Ig4}$, and $Robo2^{robo2\Delta Ig5}$, are viable, others, such as $Robo2^{robo2\Delta Ig2}$ and $Robo2^{robo2\Delta Ig3}$, are associated with lethality, showing the critical role of specific immunoglobulin (Ig) domains in development. Through examination of Robo2 variants, we analyzed midline crossing frequencies and axon guidance anomalies, finding significant deviations in midline crossing rates among lethal mutants. These findings enhance understanding of Robo-Slit interactions and highlight the functional importance of Robo2 Ig domains in both axonal pathfinding and CNS development.

628S **Impact of Na+/H+ Exchanger Proteins on Glial Function in Brain Development** Isabella Maag, Megan Wilson, Beverly Piggott University of Montana

Na+/H+ exchangers (Nhe) are major regulators of pH in the central nervous system. Human mutations in Nhe proteins cause developmental disorders. Yet, their physiological relevance to developmental processes is not well defined. Humans have 9 Nhe proteins while Drosophila melanogaster only have 3 giving a simplified system to study Nhe proteins during development. We find that knockouts of Nhe1-3 results in reduced larval brain size. A reduction in developing brain size could be due to proliferation defects and/or cell death. Subtypes of glia throughout the CNS act to regulate development by providing trophic factors to Neuroblasts which are major drivers of proliferation expanding both neuron and glial numbers. To determine whether Nhe proteins might be important in glial cells for brain development we performed RNAi knockdown of nhe2 or nhe3 in all glia and found this led to an overall reduction in brain size, glial cell number and mitotically active cells within the developing larval brain. Continued investigation into the various subtypes of glia is needed to determine the glial subtypes that contribute to these findings. This data indicates that glial pH may be important for neurogenesis. Defining the role Nhes play in development represents an important step towards elucidating the function of Nhe proteins in the brain and understanding how their dysfunction can cause developmental disorders.

629S **Homeodomain transcription factors contribute to neuronal identity in the central complex** Derek Epiney[1,2], Chris Q Doe[2,3] [1]University of Oregon, [2]Howard Hughes Medical Institute, [3]Biology, University of Oregon

Complex behaviors require neural diversity and connectivity. However, it is not fully understood how neural progenitors generate thousands of unique neurons. One important factor in this process are the homeodomain transcription factors (HDTFs). HDTFs are highly conserved from fly to worm to vertebrates and have been shown to be essential in directing neural fate decisions. HDTFs have been thoroughly studied in *Drosophila*, where the genome encodes just over 100, with many being expressed in the CNS. Here I investigate the role of several HDTFs in specifying neuronal identity in the Drosophila central complex (CX). The CX is responsible for celestial navigation as well as spatial memory formation and retention. The CX is generated by a small pool of neural stem cells (neuroblasts; NBs) called Type 2 NBs (T2NBs), whereas the majority of the remaining neurons are generated by Type 1 NBs. T2NBs undergo ~50 asymmetric division to self-renew the T2NB and give rise to an intermediate neural progenitor (INP), similar to primate outer subventricular zone progenitor. INPs undergo ~6 asymmetric divisions to self-renew and bud off a GMC, which then divides into two mature neurons. Thus, neurons born from TIINBs arise from two temporal axes: the early to late NB axis and the early to late INP axis. I will be looking at several subtypes of neurons in the CX, including P-ENs and E-PGs. These neurons are named for the neuropils in which they have connections from axons to dendrites. So, P-ENs have axons in the PB and dendrites in the EB and noduli and E-PGs have axons in the EB and dendrites in the PB and gall. We used scRNAseq to get the HDTF expression profiles of these neurons. To test the extent of which HDTFs contribute to neuronal identity, we will be used RNAi to knockdown HDTFs in each population of neurons. For example, when we knocked down Cut in P-ENs we found less axon targeting in the ellipsoid body and noduli. When we knocked down Lim1 in E-PGs we found less of these neurons in the brain.

630S **Characterization of Adiposyn, a possible fat-to-synapse inter-organ factor** Carlie Widdison[1], Josh Rosswork[1], Edward Dominquez[2], Ruben Andres[1], Dhruti Patel[1], Mia Krause[1], Edward Wang[1], Justin Bosch[1] [1]Human Genetics, University of Utah, [2]University of Illinois Urbana-Champaign

Organs communicate with each other by secreting proteins, such as hormones, into the bloodstream. Our lab has been using AlphaFold, a tool that predicts protein-protein interactions, to discover novel hormone-receptor interactions. One such discovery we have made is Adiposyn, a protein released by adipose tissue, is predicted to bind to synaptic cell-adhesion proteins called Neuroligins. We are currently validating this prediction by testing whether Adiposyn binds Neuroligins *in vivo* and exploring its potential role in synaptic function using *Drosophila* as a model organism. We have generated Adiposyn knockout flies and are investigating if they have defective synaptic function. Additionally, we are determining localization of the Adiposyn protein and where the endogenous Adiposyn gene is expressed, while also conducting co-immunoprecipitation experiments to test for Adiposyn and Neuroligin interaction *in vivo*. If this predicted interaction is successfully validated, then it would suggest that AlphaFold could be a valuable tool to detect novel ligand-receptor interactions. This would be a significant advancement in the field, as uncovering inter-organ protein interactions could reveal potential therapeutic targets if they are found to play a role in disease.

631S **Projectin regulates the growth and development of Drosophila larval muscle** Collin W. Clark, Hayden Dalton, Kiel Ormerod Biology, MTSU

The three types of muscle; skeletal, smooth, and cardiac, are essential for the complex motor functionality of humans. The most abundant type of muscle cells in animals are skeletal muscle, comprised of repeating structural units called the sarcomere. The sarcomere is composed of many proteins which contribute to its overall function. The two main proteins associated with ATP-mediated muscle contraction are myosin and actin; however, there are numerous important proteins critical to the structure and function of sarcomeres and muscles. Aldous Huxley himself recognized that certain aspects of muscle functionality deviated substantially from the biophysical limitations feasible if the sarcomere was comprised of simply actin and myosin. Our lab recently investigated the impacts of altered sallimus expression, a Drosophila ortholog of titin in single body wall muscle of third instar larvae (Michael et al, 2024). Altered sls expression in muscles lead to altered morphology of the muscle fibers including size/shape and motor neuron innervation, changes in locomotion, a reduction in contractile force, and changes in electrophysiological readings. Here we exploit these techniques to examine the other putative homologue of titin, projectin, in regulating growth and development of larvae. Altering the expression of projectin using RNA interference (UAS-RNAi-bt) using strong (MEF2-Gal4) or weakly (24B-Gal4) expressing pan-muscle drivers resulted in embryonic lethality. However, knocking down projectin in a single muscle fiber in each abdominal hemisegment had no significant impact on larval growth or gross morphology (larval length, width). Ultrastructural examination of effected muscles revealed severe impacts on the structure of sarcomeric assembly. These effects also translated to effects observable in neuromuscular junction formation and function. Our experiments further elucidate the role of projectin by examining its impacts on excitation-contraction coupling and motivated rhythmic locomotory behavior.

632S **A Better than Basic Understanding of pH: Sodium Proton Exchangers (Nhes) Regulation of Neural Development** Asher Swan Adams[1], Bernice C Lin[2], Isabella Maag[2], Ashley Bielawski[3,4], Erin Santana[2], Beverly Piggott[4] [1]Division of Biological Sciences, The University of Montana, [2]Division of Biological Sciences, University of Montana, [3]University of Michigan, [4]University of Montana

Electrolyte balance and maintaining physiological pH are vital for cell survival across the animal kingdom, especially in the brain where ionic distribution underlies neuronal function. While cellular pH is traditionally believed to remain tightly regulated (around 7.2 to 7.4), recent findings indicate significant variations over time and between cell types. pH levels, influenced by H^+ ions, profoundly affect molecular interactions, impacting cellular activities. Despite the ability of cells to adjust pH for signaling and behaviors, little is known about the role of pH in development. Major pH regulatory proteins like Na^+/H^+ exchangers (Nhe) play crucial roles in nervous system development. The human genome encodes 9 Nhe proteins and mutations in these genes cause Christianson syndrome and are linked to epilepsy and Autism Spectrum Disorders among others. Our research focuses on understanding pH regulation in neural development using the fruit fly, *Drosophila melanogaster*, as a model. Our preliminary work links distinct pH states to cell fate and identified a role for one of the three fly Nhe proteins, Nhe2, in neural stem cell proliferation. Nhe2 regulates cytosolic pH, but other family members are predicted to regulate organelle pH. We aim to elucidate how the other classes of Nhe proteins, Nhe1 (predicted Golgi localization) and Nhe3 (predicted endosomal localization) influence neural stem cell development. This research will provide fundamental insights into pH-sensitive processes crucial for nervous system formation that when disrupted, can cause disease.

633S **Transcriptional complexity in the insect central complex: single nuclei RNA sequencing of adult brain neurons derived from type 2 neuroblasts** Gonzalo N Morales Chaya[1], Derek Epiney[2], Noah Dillon[2], Chris Q Doe[2] [1]University of Oregon, [2]Biology, University of Oregon

In both invertebrates such as *Drosophila* and vertebrates such as mouse or human, the brain contains the most diverse population of cell types of any tissue. It is generally accepted that transcriptional diversity is an early step in generating neuronal and glial diversity, followed by the establishment of a unique gene expression profile that determines neuron morphology, connectivity, and function. In *Drosophila*, there are two types of neural stem cells, called Type 1 (T1) neuroblasts and Type 2 neuroblasts (T2). In contrast to T1 neuroblasts, T2 neuroblasts generate Intermediate Neural Progenitors (INPs) that expand the number and diversity of neurons. The diversity of T2 neuroblast-derived neurons contributes a large portion of the central complex (CX), a conserved brain region that plays a role in sensorimotor integration, including celestial navigation. Recent work has revealed much of the connectome of the CX, but how this connectome is assembled remains unclear. Mapping the transcriptional diversity of T2 neuroblast-derived neurons, including those projecting to the CX, is a necessary step in linking transcriptional profile to the assembly of the connectome. Here we use single nuclei RNA sequencing of T2 neuroblast- derived adult neurons to identify over 150 distinct cell clusters. We map neurotransmitter and neuropeptide expression and identify unique transcription factor combinatorial codes for each cluster (presumptive neuron subtype). This is a necessary step that directs functional studies to determine whether each transcription factor combinatorial code specifies a distinct neuron type within the central complex. We map several well- characterized columnar neuron subtypes to distinct clusters, and two neuronal classes map to a single cluster. Our data support the hypothesis that each cluster represents a one or a few closely related neuron classes.

634S **Glial expression of ER membrane protein complex subunit 4 (EMC4) plays a role in survival, development, and larval behavior** Inés Riojas, María José Orozco Fuentes, Otoha Tatami, Salma Abdelkhalek, Monique Dirzo, Dr. Rebecca Delventhal Department of Biology, Lake Forest College

The endoplasmic reticulum (ER) is involved in the modification, packaging, and insertion of membrane proteins in the cell. The EMC is a membrane protein complex in the endoplasmic reticulum composed of 8-10 subunits that work together to facilitate protein biogenesis. Our lab observed that *Drosophila melanogaster* exhibited shorter lifespans of 5-6 days instead of 2-3 months when an RNAi knockdown of EMC subunit 4 was induced in glia. These flies also exhibited severe locomotion impairments (weakened climbing), an increase in protein aggregation, a decrease in developmental viability, and delayed development. Through a detailed developmental analysis, we observed approximately a one-day delay per stage: wandering 3rd instar larva, pupation, and eclosion, suggestive of a delay that begins in larval development. To test other larval phenotypes possibly affected by the knockdown, we examined olfactory-driven behavior as chemosensory perception is crucial to their survival. Preliminary analyses indicate there are no significant differences in olfactory sensitivity between larvae with and without the glial knockdown of EMC4, though studies are ongoing. These results suggest that the observed developmental delay may be due to other alterations in glial function besides olfaction. To determine whether the developmental role of EMC4 impacts the adult survival phenotype, we conducted a temporally restricted glial EMC4 knockdown using a temperature-sensitive Gal80 inhibitor of the UAS-Gal4 system. We observed that the adult-specific knockdown did not exhibit the same severity of survival or locomotion declines, further supporting the importance of EMC4 in development. Our overall findings suggest EMC4 has a key role in protein biogenesis, which leads to severe phenotypes in locomotion, survival, and development when knocked down in glia.

635S **Neuropile Ensheathing Glia Modulate Seizure Susceptibility of *Drosophila melanogaster*** Sarah Iannone, Mar Hinestroza, Kayla Bortlik, Lexis Grandel, Claire Sipes, Dionna DeFazio, Alexis Hill College of the Holy Cross

The nervous system must rapidly and effectively adapt to environmental challenges, such as fluctuations in temperature. Homeostatic processes allow organisms to maintain critical behavioral and physiological stability under environmental stress. Here we demonstrate that in *Drosophila melanogaster*, a specific glial cell type, neuropile ensheathing glia (EGN), plays a significant role in regulating the nervous system, as genetic manipulations targeted to EGN influence susceptibility to heat-induced and bang-induced seizure susceptibility assays in adult flies. More specifically, our lab has found that EGN knockdown of the voltage-gated potassium channel gene *seizure* (*sei*) and the sodium/potassium/chloride transporter *ncc69* increases seizure susceptibility. Studies utilizing a temperature-sensitive *Gal80* revealed that *sei* and *ncc69* function developmentally in EGN to affect adult fly seizure susceptibility, with *sei* functioning specifically during pupation. While EGN have been previously implicated in phagocytic functions, our preliminary results indicate that manipulations of the engulfment receptor *draper* do not affect seizure susceptibility, suggesting a different mechanism. Through current studies examining the developmental functions of *sei* and *ncc69* in EGN, we aim to uncover whether their regulation of seizure susceptibility operates independently of phagocytosis or reveals a relationship between phagocytosis and neural excitability. Our findings highlight developmental functions of glia in maintaining neural stability, providing insights into how glial cells modulate the nervous system.

636S **Identifying targets of the temporal transcription factor Hunchback in postmitotic neurons** Benjamin Brissette, Chris Q Doe Institute of Neuroscience, University of Oregon

Neural stem cells known as neuroblasts in *Drosophila melanogaster* divide and differentiate to form the approximately 100,000 neurons of the nervous system. One of the means by which they achieve the required diversity of neuron types is through the expression of a sequence of temporal transcription factors (TTFs). The offspring of a neuroblast expressing a particular TTF continue to express that TTF, though the function of these transcription factors in postmitotic neurons is unknown.

Hunchback (Hb) is an early TTF that is necessary and sufficient to specify early-born neuronal fate. Previous work in our lab has shown that Hb targets differ between neuroblasts when they are expressing this transcription factor, but the targets of Hb binding in postmitotic neurons and their potential roles in axon guidance and synaptic targeting have not been studied. To identify postmitotic Hb targets, I use Cleavage Under Targets & Release Under Nuclease (CUT&RUN) in the lineage of the 7-1 neuroblast after the early-born neurons that continue to express Hb have arisen.

Hunchback and Castor (Cas) are early and late TTFs in the 7-1 lineage, respectively, and the postmitotic neurons that arise from these neuroblast windows are distinct. In spite of this, Hb and Cas have the same *in vitro* DNA-binding specificity. To determine whether Hb and Cas targets differ *in situ*, I use CUT&RUN to identify Cas targets in comparison to Hb targets, both in the lineage of the 7-1 neuroblast.

Additionally, Hb continues to be expressed in postmitotic neurons long after their birth and after many features of neuronal identity have been determined. Other work in our lab is investigating the role of Hb in these postmitotic neurons, so CUT&RUN is again utilized in the 7-1 neuroblast lineage at later timepoints to examine how the role of Hb changes over developmental time within the same postmitotic neurons.

In summary, while Hb is well-known for its role in specifying early-born neurons, the function of its continued expression in postmitotic neurons has remained unclear. The use of CUT&RUN in the lineage of a specific neuroblast, 7-1, allows us to understand how Hb determines neuron type, the role of its continued expression in postmitotic neurons, and how it differs from other TTFs such as Cas.

637S **Characterization of genes in the Ddc gene cluster of Drosophila for Dopamine-dependent behaviors** Rhea Mistry[1], George Blue[1], Sandra Watson[2] [1]Department of Natural Sciences, Pitzer College, [2]Scripps College

In both humans and fruit flies, neuronal synthesis of dopamine (DA) occurs via a two-step process: tyrosine is converted to L-Dopa in the rate-limiting step mediated by the enzyme tyrosine hydroxylase (TH, also known as pale in flies) and L-dopa is subsequently converted to DA by the enzymatic action of dopa decarboxylase (Ddc). Interestingly in flies, the gene encoding Ddc is found in a cluster of 18 functionally related genes whereby isolated mutants display defects in cuticle formation, hardening, melanization and/or disturbed catecholamine metabolism. Mutations in seven of the genes in the Ddc gene cluster display increased levels of DA and its derivatives at the pupal stage but how their molecular functions influence DA metabolism and effect adult DA-dependent behaviors remains unknown. We aim to characterize two mutants in the Ddc gene cluster, *mindbomb (mib2) and brain tumor (brat),* for their role in DA metabolism and DA-dependent behaviors. To do this we use imaging, genetic, and behavioral techniques to assay DA neuron number, sleep and circadian phenotypes, and survival under conditions that increase DA. Our preliminary data show that knockdown of mib2 pan-neuronally is sufficient to increase night activity and activity on a precursor for DA (L-dopa) compared to control, pointing to possible defects in DA breakdown. Conversely, knockdown of brat pan-neuronally leads to blunted night activity on food supplemented with L-dopa. Future experiments aim to validate these results and to determine whether these genes play a role in the health of DA neurons. Both genes have a corresponding functional human ortholog, thus investigating novel ways in which DA metabolism is regulated has the potential to translate to the human neurological disorders.

638S **Evolutionary conservation of Slit-Robo signaling in flies and mice** Savannah Beaupre[1], Tim Evans[2] [1]Biological Sciences, University of Arkansas, [2]University of Arkansas

The evolutionarily conserved Slit-Robo signaling pathway regulates axon guidance during embryonic development in bilaterian animals. Slit ligands are expressed at the midline of the developing nervous system and serve as a repulsive signal for Robo-expressing axons, preventing inappropriate midline crossing. In *Drosophila*, there is a single *slit* gene, while mammals have three (*Slit1, Slit2, Slit3*). The binding of Slit to Robo appears to be well-conserved across species: fly Slit can bind to vertebrate Robos and vice versa. We have previously found that mouse Robos (mRobo1 and mRobo2) can partially rescue midline repulsion in *Drosophila robo1* mutants. Here we examine the ability of mouse Slit (mSlit2) to substitute for fly Slit to regulate the midline crossing of axons during *Drosophila* embryonic central nervous system development.

Using CRISPR/Cas9-mediated gene replacement, we replaced the *Drosophila slit* gene with mouse Slit2 and examined axon guidance in these modified embryos. We observed partial rescue of midline repulsion in homozygous $slit^{mSlit2}$ embryos, indicating that mouse Slit2 can activate midline repulsive signaling of *Drosophila* Robo receptors, though not as effectively as fly Slit. This suggests that the mechanisms of Slit-Robo pathway activation are conserved in insects and mammals.

639S **Investigating Locomotor Behavior in *Drosophila Nckx30c* Mutants** Sophia Bourgeois[1], Nahian Majlish[1], Victoria Hand[2], Selene Tan[2], Atulya Iyengar[2], Stanislava Chtarbanova[2] [1]Biological Sciences, The University of Alabama, [2]The University of Alabama

Drosophila melanogaster is a well-suited model organism for studying the pathogenesis of human diseases including neurological conditions such as seizures and neurodegeneration. Our lab has identified a mutant in the gene *Nckx30c*, which encodes a potassium (K^+)-dependent sodium/calcium (Na^+/Ca^{2+}) exchanger predominantly expressed in brain tissue and is orthologous to mammalian Solute Carrier Family 24 (SLC24). In comparison to *wild type* controls, *Nckx30c* mutants exhibit temperature-sensitive (TS)-paralysis and age-dependent neurodegeneration, which is also accompanied by decreased lifespan. Mutants with neurodegenerative phenotypes often display locomotor defects, however, in the case of *Nckx30c* flies, these have not been investigated. To expand the characterization of *Nckx30c* mutants, here we investigate the locomotor behavior of both mutant alleles and fly lines in which the *Nckx30c* gene was knocked down (KD) specifically in neurons or glia. Locomotor behavior was assessed using an automated video tracking system equipped with a heated plate to reveal temperature-sensitive phenotypes. Measurements were carried for both 5-days-old (young) and 30-days-old (aged) flies, accounting for potential sex differences. Our results show that in comparison to *wild type* flies, mutants of both sexes carrying two *Nckx30c* alleles display increased temperature sensitivity resulting in abnormal locomotor behavior characterized by reduced velocity and lower percent active time. These phenotypes were exacerbated with age. Neuronal KD of *Nckx30c* in males and females phenocopied these results. Currently, we are finalizing data collection to increase sample size and perform statistical analyses. Overall, our data indicate that *Drosophila Nckx30c* plays a role in locomotor behavior. Because the role of its ortholog SLC24 in the brain is not fully understood, our results could potentially provide novel insights about the function of this gene in humans.

640S **Development of Post-embryonic Neural Circuits From a Lineage-based Approach** Marianne Maughan, Erin Beck, Haluk Lacin University of Missouri - Kansas City

During nervous system development, individual stem cells divide and give rise to a population of neurons called a lineage. There are 34 lineages in the ventral nerve cord (VNC) of Drosophila melanogaster (fruit fly), which is analogous to the vertebrate spinal cord. The adult nervous system is built during metamorphosis from neurons of these lineages. Leveraging genetic tools available in the fruit fly, I aim to investigate the molecular mechanisms driving the development of lineage 10B neurons within the adult nervous system. Two recently published datasets have made the fly VNC a unique model system to study neural circuit development: single cell RNA sequencing (scRNAseq) provides transcriptome profiles for individual neuronal lineages and Electron Microscopy (EM) volume provides synaptic connectivity among neurons at a single synapse resolution. EM volume identifies a group of proprioceptive sensory neurons, called the femoral chordotonal (FeCo) neurons, that make strong synaptic connections with 10B neurons. scRNAseq analysis identified a set of key genes likely involved in cell-fate acquisition, axon guidance, and synapse formation of 10B neurons.

Using this information, I will perform gain or loss of function experiments to elucidate which transcription factors are essential for 10B identity and which cell-to-cell communication molecules are needed for 10B neurons to integrate into neuronal circuits, particularly those with the FeCo neurons. Preliminary results indicate that the 10B lineage has approximately 72 neurons per neuromere. We have identified 3 distinct subsets of 10B neurons within the scRNAseq data set. We are currently working to identify essential transcription factors and key guidance molecules from the scRNAseq datasets. Our research will lead to the understanding of molecular pathways underlying cell fate and lineage identity of 10B neurons and the circuits they form. Moving forward, we will manipulate components of this system to better understand circuit formation.

641S **Temporal Transcription Factors Control Neural Fate Specification through Regulation of Chromatin Accessibility in *Drosophila* Medulla Neuroblasts** Tejus Sreelal, Yu Zhang, Hailun Zhu, Xin Li Univ. of Illinois Urbana-Champaign

During development, *Drosophila* neuroblasts are temporally patterned by the sequential expression of Temporal Transcription Factors (TTFs) to generate a great diversity of neural types in a birth-order dependent manner. However, it is not clear how these TTFs expressed in neuroblasts control the fate of the progeny because in many cases TTFs are not inherited in the progeny. We use the *Drosophila* medulla, part of the visual processing center, to address this question. We and others identified a series of TTFs that are sequentially expressed in *Drosophila* medulla neuroblasts as they age and control their temporal patterning. We hypothesized that TTFs regulate the dynamic changes in chromatin accessibility to control temporal patterning and neural fate specification. We profiled chromatin accessibility and gene expression in the same single nuclei using FACS sorted medulla neuroblasts of different ages and their progeny. Our data revealed dynamic changes of chromatin accessibility during temporal patterning of medulla neuroblasts and in their progeny. Through analyzing the differentially accessible regions (DARs) linked with gene expression, we identified and cloned possible enhancers controlling the expression of genes encoding TTFs and neural TFs. Using the T1 neuron, specified by the transcription factor code Ets65A, Oc and Sox102F, as an example, we verified that the enhancers of neural TF genes drive correct expression patterns and identified TTF binding sites within these enhancers. To further test our hypothesis that TTFs regulate the chromatin accessibility during temporal patterning, we performed single-nuclear ATAC-seq on FACS sorted medulla cells with each of the TTFs knocked out, and examined how loss of each TTF affects the dynamic changes in chromatin accessibility. Our results demonstrate that specific TTFs are required to open up enhancers of neural TFs to control neural fate.

642S **Teneurin-mediated synaptic organization occurs via the cytoskeletal adaptor CAP** Juan Carlos Duhart, Benjamin Seitz, Jesse Humenik, Tim Mosca Neuroscience, Thomas Jefferson University

Signals that influence how synaptic connections in the brain begin by connecting the cell surfaces of partner neurons. Transmembrane proteins connect presynaptic neurons to postsynaptic targets, but must also engage downstream intracellular partners to ensure the growth, strengthening, and persistence of that connection. One major family of transmembrane proteins, the Teneurins, acts as a key regulator of multiple steps of synaptic development, maturation, and organization but how Teneurins transduce signals via downstream cellular machinery remains unknown. A grasp of the downstream mechanisms is key to understanding how synapses form, and importantly, how cell surface proteins regulate systemic processes in the rest of the cell. We discovered that CAP, the Cbl-associated protein that interacts with the cytoskeleton and typically localizes to focal adhesions, is a synaptic protein required for development. CAP localizes pre- and postsynaptically at developing neuromuscular synapses and its expression overlaps with presynaptic Ten-a and postsynaptic Ten-m. In the absence of *CAP*, developing synapses display multiple defects: NMJ width, length, and bouton number are all reduced, indicating CAP is essential for synapse growth. Loss of *CAP* also regulates active zone density and morphology as imaged by stimulated emission-depletion (STED) microscopy, and impairs the normal apposition of active zones by postsynaptic glutamate receptors. Further, we also observed defects in synaptic maturation in *CAP* mutants including more ghost boutons (regions of presynaptic neuronal membrane that lack apposite postsynaptic scaffolding), decreased levels of postsynaptic proteins including α-spectrin and Hts, and impaired presynaptic microtubule organization. We find, using tissue-specific rescue experiments in *CAP* mutants, that CAP is required in both presynaptic motoneurons and postsynaptic muscle to organize synapses, suggesting cell-autonomous and non-cell-autonomous roles. *CAP* mutants nearly completely phenocopy *ten-a* mutants at the synapse and we find that *CAP* and *ten-a* function in the same genetic pathway to regulate synaptic organization, suggesting Ten-a functions via CAP to regulate synaptic development. We also find that a role for CAP in regulating synaptic organization is conserved in the central nervous system as *CAP* loss in adult brain olfactory neurons increases active zone number. Intriguingly, this result phenocopies effects of *CAP* loss on NMJ active zones, but deviates from *ten-a* phenotypes, suggesting CAP also has Teneurin-independent functions in synaptic development. Altogether, this highlights that CAP is essential for multiple stages of synaptic development, but also uncovers a novel mechanism connecting essential Teneurin-based cell surface signaling in synaptogenesis with downstream machinery to transmit the signal to the rest of the neuron.

643T **Impacts of Tissue Structure on Chemoattractant Signaling in the Ovary** Alexander George[1], Naghmeh Akhavan[2], Bradford E. Peercy[2], Michelle Starz-Gaiano[1] [1]Biological Sciences, University of Maryland, Baltimore County, [2]Mathematics and Statistics, University of Maryland, Baltimore County

Proper morphogenesis requires subsets of cells to move to new locations at certain times. How this is precisely controlled in a dynamic and heterogenous environment is not well understood. To investigate directed cell migration, we study the border cells of the ovary, which arise in the anterior follicular epithelium and migrate to the oocyte. Prior work has identified physical and chemical cues that are important to this process. We sought to determine how these separate cues impact each other to direct migration. Through live imaging analysis, we observed stereotyped variations in cell migration speeds along the migratory path that correlated with less restrictive physical domains between the substrate cells, the nurse cells. These speed variations are dampened by overexpression of chemoattractant, suggesting that the cell behaviors are influenced not just by physical constraints but also by chemoattractant concentrations. We then developed a mathematical model to characterize the effect of variable physical features on chemoattractant distribution and migratory behaviors. Results from this modeling showed dramatic drops in the chemoattractant gradient in some regions, and indicated that this is sufficient to explain the observed changes in cell movement speeds. To investigate this in more detail, we examined mutant cases that had fewer but larger spaces between substrate cells. Mutant egg chambers with larger regions between cells could rescue incomplete migration in response to chemoattractant overexpression, supporting our idea that the physical spaces between cells alter chemical distribution and migratory behaviors. To further test this hypothesis, we are currently examining the distribution of chemoattractants in the egg chamber. Our data suggest that tissue structure has a key role in shaping chemoattractant gradients and determining migratory cell behaviors.

644T **Detection of novel short linear motifs in *Drosophila* transcription factors involved in segmentation** Minh Le, Leslie Pick University of Maryland

The segmented body plan is a characteristic shared across all insects. Despite ultimately giving rise to same phenotype, the underlying genetic network involved in segmentation, originally identified in *Drosophila melanogaster* (Nusslein-Volhard & Wieschaus 1980), is surprisingly different across different species of insects. This diversity is often attributed to changes in cis-regulatory elements controlling expression of transcription factors (TF) involved in segmentation. However, the TF Fushi tarazu (Ftz) exemplifies a case where the change in genetic network is associated with the evolution of short linear motifs (SLiMs), short amino acid chains in a protein that confer function(s). Specifically, during evolution, in lineages leading to *D. melanogaster,* Ftz lost the homeotic YPWM SLiM and gained the LXXLL SLiM. This facilitated a novel interaction between Ftz and the orphan nuclear receptor Ftz-F1, allowing Ftz to function as a pair-rule TF (Lohr et al., 2001; Lohr & Pick, 2005; Heffer et al., 2010). However, this is the only well-documented case of SLiM evolution involved in segmentation. To determine if this phenomenon applies to other TFs in addition to Ftz, we used MEME, a sequence alignment-based motif discovery tool (Bailey & Elkan, 1994), to computationally predict evolutionarily derived SLiMs in other *D. melanogaster* segmentation TFs. So far, unique, conserved but evolutionarily variable regions were detected in *D. melanogaster* segmentation TFs Runt, Hairy, and Knirps. Particularly, regions that are consistently conserved in Diptera, but not other order of insects were detected, suggesting that these are SLiMs evolved in Diptera. Functional tests using GAL4-UAS were employed to determine if predictions by MEME represent functional SLiMs, comparing the phenotype of embryos expressing wildtype vs mutant proteins. Of the tests performed to date, an LXXML SLiM in the pair-rule TF Runt is the most interesting. The LXXML is consistently present in Diptera, but not in other insects or in the mammalian homolog RUNX1. Preliminary results suggest that this SLiM is involved in abdominal segmentation, attributed previously to a gap gene-like function of Runt during early development (Tsai & Gergen, 1994). Our work seeks to determine whether changes in protein sequences, specifically changes in SLiMs, contributed to the evolution of segmentation networks. Future experiments involve testing MEME predictions by endogenous mutations via CRISPR-Cas9.

645T **Identifying candidate pH-sensitive proteins that regulate tissue growth** Laura Martins[1], Madelaine Surette[1], Ramy Wong[2], Daniel Orozco[2], Bree Grillo-Hill[2] [1]Biology, San Jose State University, [2]San Jose State University

Intracellular pH (pHi) is tightly regulated by cells, and emerging evidence suggests that regulated pHi dynamics modulate distinct cell behaviors. Cancer cells have constitutively increased pHi, which we and others have shown alters functions of pH-sensitive proteins leading to altered cell behaviors. To regulate pHi, cells use a variety of ion exchangers and acid loaders/extruders to maintain pH near physiological levels. *NHE1* is an ubiquitously expressed sodium proton exchanger that acts as a rheostat to maintain physiological pH. Our lab generated transgenic flies that inducibly express *DNhe2*, the homolog of *NHE1*, in the *Drosophila* eye. One goal of our lab is to identify specific pH sensitive proteins that are dysregulated at an increased pHi. We previously showed that overexpression of *DNhe2* results in a rough eye phenotype, and is sufficient to increase pHi, increases cell proliferation in vivo, and paradoxically increases autophagic cell death. Here we describe a reverse genetic screen to identify candidate pH-sensitive proteins. We screened a collection of 193 *Drosophila* lines covering 94% of the second chromosome. We visually inspected flies for enhancement or suppression of the rough eye phenotype. We identified 35 regions of the second chromosome that show an interaction with *DNhe2*. We focused on one region defined by two overlapping deficiencies that both suppressed the *DNhe2* rough eye phenotype, Df(2L) ED1303 and Df(2L) ED1315, spanning 38B4-38C6. We identified 8 candidate genes in the region defined by the overlapping deficiencies. We obtained genetic reagents to alter expression of each gene, and tested them for suppression of *DNhe2*. Loss-of-function alleles of one gene, *CG10949*, suppressed the *DNhe2* rough eye phenotype, similar to the original deficiencies. *CG10949* is a novel uncharacterized gene, with predicted homology to MADF transcription factors. The only publication on the function of *CG10949* suggests a role in regulating growth in the *Drosophila* wing. Our current work includes characterizing the retinal phenotype of *CG10949*, and elucidating the genetic interaction with *DNhe2*. Understanding the effects of increased pHi and which pH sensitive proteins can help us understand and uncover therapeutic targets.

646T **Me31B is required during indirect flight muscle myogenesis in *Drosophila*** Mutiat Abdulkareem[1], Harry Manning[2], Ming Gao[3], Maria L Spletter[1] [1]Biological and Biomedical Systems, University of Missouri Kansas City, [2]University of Bath, [3]Indiana University Northwest

During muscle development, RNA regulation determines the balance in isoform expression of sarcomere proteins, enabling muscles to fine-tune their morphologies and contractile properties. RNA-binding proteins (RBPs) direct alternative splicing to produce multiple isoforms of myofiber-type specific proteins and regulate nuclear export, trafficking, stability, and translation dynamics within the muscle cell. We have previously shown roles for CELF and RBFOX family RBPs in *Drosophila*, helping to establish the indirect flight muscles (IFMs) as a powerful model to study RBP function in muscle development. Here we identify a role for the DEAD-box RNA-helicase Me31B in adult myogenesis. Me31B is the homolog of vertebrate DDX6, which is found in P-bodies and stress granules and functions both in translation suppression and mRNA degradation. DDX6 is associated with intellectual disability, dysmorphic facial features, feeding difficulties, and mild cardiac defects. In flies, Me31B has been reported to localize to P-bodies and mediate repression of maternal transcripts through translation repression and regulation of mRNA stability in the fly embryo. We show that Me31B is expressed at a high level in muscle cells and localized at the nuclear membrane in distinct punctate structures in IFMs, consistent with a role in IFM development. We determine Me31B localization with respect to the nuclear pore complex, cap-binding protein and translation factor eIF4E, and P-body marker Tral. We further test if Me31B localization is Bru1-dependent in IFM, as Me31B, eIF4E, Tral, and Bru1 are part of a complex that mediates translation repression in oocytes. Knockdown of *me31B* with muscle-specific drivers Mef2-Gal4, Act88F-Gal4, and UH3-Gal4 results in pupal lethality. Structure-function analysis using CRISPR alleles targeting distinct Me31B domains reveals that a single helicase domain is sufficient to support muscle development but disrupts the punctate pattern of Me31B accumulation at the nuclear membrane. Our data reveal a role for DDX6 homolog me31B in pupal muscle development, identifying another RBP with a function in muscle and emphasizing the importance of RNA regulation during myogenesis.

647T **The Osiris family genes regulate the tube maturation process in the *Drosophila* trachea** Niraj Dhakal[1], Katie Birstow[1], Klejvia Sejdini[1], Lana Skrna[1], John Pirau[1], Lan Jiang[2] [1]Biological Sciences, Oakland University, [2]Oakland Univeristy

Drosophila trachea is a premier model to study the development of tubular organs, such as the human lung, blood vessels, and kidneys. It is a ramifying network of epithelial tubes with a monolayer of epithelial cells surrounding an apical lumen. After the formation of continuous tubes, tube maturation follows. Tracheal tube maturation starts with an apical secretion pulse that deposits apical extracellular matrix (aECM) components to form a chitin-based luminal aECM. Following tube expansion, this transient luminal aECM is cleared by endocytosis. The permanent aECM, known as «taenidial folds,» is produced to form a ring-like structure running perpendicular to the tube length and subsequent air filling can occur.

The *Osiris* (*Osi*) gene family is located at the *Triplo-lethal* (*Tpl*) locus on chromosome 3R 83D4-E3 and exhibits dosage sensitivity. Protein sequence analysis of Osi proteins identified an endo-lysosomal signal sequence and a domain of unknown function (DUF1676). This finding indicates the potential roles of Osi proteins in protein trafficking and other unidentified cellular functions. We show that several *Osi* genes are highly expressed in the *Drosophila* trachea. In addition, *Osi* genes function redundantly to regulate tracheal tube maturation, including the stability of cellular junction, the organization of taenidial folds, the integrity of the tracheal tubes, and the air filling of the tubes. Nonetheless, *Osi* genes are not required for the formation and clearance of the transient luminal aECM. In summary, the *Drosophila Osi* genes play an important role in developing mature tracheal tubes.

While no clear *Osi* homologs exist in mammals, a homology search identified sequence similarity between *Osi* genes and the mammalian *Glyoxalase 1* (*Glo-1*) gene. Reduced *Glo-1* is linked to tube morphology defects, such as narrowing in atherosclerotic arteries. These findings imply that *Osi* genes may have overlapping roles with *Glo-1* or other functional homologs in shaping mammalian tubular organs.

648T **Exploring the Complexity of the *kayak* Locus in Eye Development** Manuel Alejandro M Zúniga García[1], Juan J Riesgo-Escovar[2] [1]Universidad Nacional Autónoma de México, [2]Neruobiología del desarrollo y Neurofisiología, Universidad Nacional Autónoma de México

kayak (*kay*) is the only *fos* homolog in *Drosophila* and is required in multiple tissues and developmental stages. To investigate its role in eye development, we generated mutant clones and assessed *kay* function using optical and scanning electron microscopy (SEM) to characterize mutant eye phenotypes. Our findings show that *kay* loss-of-function mutations cause defects in imaginal structures, with phenotypic outcomes varying across alleles—some alleles produce severe defects, while others have minimal or no visible impact. Interestingly, all tested alleles share a common outcome: embryonic lethality. Together, these results highlight the complex functions of the *kayak* locus and its critical role in developmental processes.

649T **Characterization of novel *Drosophila* Egf receptor signaling targets with roles in eggshell morphogenesis** Autumn Bullek[1], Sara Delgado[2], Kayla Eckrote[1], Federico Moran[2], Megan Robinson[1], John Tondora[1], Molly Yuschock[1], Lisa Kadlec[1] [1]Biology and Earth Systems Science, Wilkes University, [2]Biochemistry, 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 previously identified potential downstream transcriptional targets of the Egf receptor using the *Drosophila* ovary as a model system. Our initial work compared gene expression in fly ovaries where the activity of the Egfr pathway was reduced (gurken mutant), wild-type (OreR), or constitutively active (CY2/λTop). We have employed a number of approaches to further investigate the expression, biological function, and mechanism of action of a subset of putative genes of interest, focusing primarily on genes of previously unknown function. A small-scale functional screen using available collections of UAS-RNAi transgenic flies and P-element insertion lines was used to investigate the possible functions of a group of these novel Egfr-responsive genes. This screen identified multiple genes activated by Egfr signaling that appear to have roles in the morphogenesis, rather than the patterning, of eggshell features. Gene mutant/knockdown phenotypes include severely decreased chorionic integrity, shortened eggs, and various dorsal appendage malformations, as well as decreased fertility. We have also used the CRISPR-Cas9 system to create mutations in some of these genes, with a goal of generating null mutant lines. Characterization of these mutants has so far revealed expected (previously observed) phenotypes, and in some cases additional eggshell phenotypes not seen with the original knockdown or P-element flies. We are continuing to evaluate our most recently created CRISPR mutants, and are also using them, as well as previously generated CRISPR lines, for further study and characterization of our genes of interest, for example by investigating possible underlying ovarian defects via fluorescence microscopy to look at ovary structure.

650T **Investigating the function of Ecdysone during dorsal closure in Drosophila embryogenesis** Jaeho Lee[1], Amanda Lee[2], Robert Ward[3] [1]Biology, Case western reserve univercity, [2]Case Western Reserve, [3]Biology, Case western Reserve Univercity

20-hydroxyecdysone (20E) is a 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 early genes such as Broad-Complex (BR-C), E74, and E75, which activate late genes performing stage- and tissue-specific developmental functions. Previous studies showed a lack of 20E signaling exhibits phenotypes in germband retraction, head involution, dorsal closure, and cuticle synthesis during embryogenesis. However, the detailed function of ecdysone during embryogenesis is not clearly understood. Here, we are focusing on the role of 20E signaling during dorsal closure in mid-embryogenesis. To gain a mechanistic understanding of the function of the 20E during dorsal closure we are characterizing phenotypes associated with loss of *disembodied* (*dib*) and *shroud* (*sro*), two “Halloween” genes that encode p450 cytochrome enzymes required for 20E synthesis. Immunostaining of *dib* and *sro* mutant embryos during dorsal closure show that JNK and Dpp signaling, which are initial signaling pathways of dorsal closure, appear normal. Likewise, the formation of the actomyosin cable on the leading edge is normal in mutant embryos. However, time-lapse imaging of Ecad and Sqh labeled *dib* and *sro* mutant embryos reveal poor formation of the canthus and zippering of the contralateral epidermal sheets. We also observe that mutant embryos show significantly slowed germ band retraction, abnormal shape and behavior of amnioserosa cells during dorsal closure, and reduced filopodia activity at the leading edge. Based on our observations, ecdysone seems to work in both the amnioserosa and epidermis for some aspects of actomyosin dynamics. We used tissue-specific expression of a dominant negative Ecdysone receptor (EcR-DN) to compare with the Halloween mutants and found that ubiquitous expression of EcR-DN shows a strong dorsal closure phenotype, while pannier-GAL4 (dorsal epidermis) driven EcR-DN has a completely penetrant, but milder dorsal closure defect. Surprisingly, amnioserosa-specific EcR-DN expression does not show a dorsal closure phenotype. To identify ecdysone-regulated genes required for dorsal closure, we will conduct RNA-seq on wild-type and ecdysone mutant embryos during dorsal closure. Also, we will conduct live imaging on tissue-specific EcR-DN expression and identify major tissues that generate dorsal closure phenotypes.

651T **Outspread, a myosin phosphatase interacting protein, determines tubular organ dimension** Ji Hoon Kim[1], Ankita Holenarasipura[2], Parama Paul[2], Deborah Andrew[2] [1]Johns Hopkins University, [2]Cell Biology, Johns Hopkins University

Epithelial tubular organs are essential for viability in all higher multicellular organisms. The optimal functionality of a tubular organ demands its proper architecture, which is achieved during development. Salivary gland (SG) development in the Drosophila embryo provides an excellent model system to study budding morphogenesis wherein a 2-dimensional (2D) epithelial sheet morphs into a 3-dimensional (3D) epithelial tube. This process is driven by morphogenetic signals acting on non-muscle myosin II (MyoII) to induce changes in cell shape and arrangement. We have searched for genetic factors that affect SG morphogenesis and found that a gene long known to affect wing placement – *outspread* (*osp*) – is important for shaping the 3D architecture of the SG. *osp* expression in the SG requires the FoxA transcription factor Fork head (Fkh), which controls SG cell invagination and tube formation. Osp is a large cytosolic protein with two pleckstrin homology (PH) domains at its N-terminus and coiled coil structures at its C-terminus. Osp is closely related to a mammalian protein – Myosin Phosphatase Rho Interacting Protein (M-RIP) – suggesting a potential function in the Rho GTPase-MyoII signaling pathway. Loss of *osp* results in shorter SG tubes with wider lumens and Osp overexpression results in longer SG tubes accompanied with strong invagination defects. Similar changes in SG tube dimensions result from perturbing MyoII function in this tissue: hyperactivation of MyoII decreases SG tube length whereas MyoII depletion generates elongated SG tubes. In invaginating SG cells, Osp forms condensate-like structures that localize to the apical medial domain; a localization that is dependent on one of its two PH domains. Condensate formation of Osp requires the intrinsically disordered region (IDR) located in the middle of the protein. Through its C-terminal coiled coil domains, Osp co-localizes and physically interacts with Mbs, the myosin phosphatase regulatory subunit, suggesting negative regulation of MyoII activity by Osp. Indeed, overexpressed Osp suppresses apical-medial MyoII accumulation in SG cells. Based on these findings, we propose that Osp recruits concentrated MyoII phosphatase to limit MyoII activity during SG invagination, thereby providing a novel regulatory mechanism for controlling organ geometry during tubular morphogenesis.

652T **Integration of cell cycle control and morphogenetic signaling during *Drosophila* salivary gland development** Jeffrey Matthew[1], SeYeon Chung[2] [1]Biological Sciences, Louisiana State University, LSU, [2]Biological Sciences, Louisiana State University

The formation of complex organs during development depends on tightly coordinated gene expression, regulation of cellular activities, and signaling cascades that govern changes in cell and tissue shape. In this study, we investigate the *Drosophila* embryonic salivary gland (SG) as a model for tubular organ morphogenesis. We reveal a novel role for the SP1/KLF transcription factor Huckebein (Hkb) in orchestrating spatial morphogenetic movements alongside cell cycle control to maintain organ size. Our findings show that proper SG development requires a Hkb-dependent, precise, distal-to-proximal endoreplication (endocycle) wave that begins as the SG initiates Rho1-dependent internalization and migration. Hkb regulates endoreplication in the SG by repressing transcription of key cell cycle and pro-apoptotic genes. Altered cell cycle results in abnormal cell death, leading to the formation of a small organ size. Using the Fly-FUCCI genetic tool and EdU labeling alongside genetically manipulated Rho1 and Rac1 activity within the SG primordia, we further show that disrupted Rho1 signaling disrupts progressive endoreplication in the SG. These results highlight the role of Rho1 and Rac1 in shaping SG morphogenesis and suggest that the initiation of endoreplication may depend on SG tissue shape changes or Rho1 signaling. Interestingly, we observe altered cell cycle dynamics when FUCCI is overexpressed in the SG using the UAS promoter, compared to the FUCCI system with a ubiquitous promoter. This suggests that caution is required when using the FUCCI system under the UAS promoter. Our findings underscore the interconnectedness of cell cycle regulation and morphogenetic movements, shedding light on how these processes are integrated to shape developing organs.

653T **gilgamesh, Drosophila casein kinase 1γ, is required for myosin-dependent junction strengthening and epithelial folding** Reina E Koran, Lingkun Gu University of Nevada Las Vegas

Adherens junctions, the Cadherin-based cell-cell junctions that resist physical tension, often mediate mechanosensitivity during morphogenesis. During the internalization of Drosophila mesoderm, puncta-like spot adherens junctions serve as the anchors to connect contractile actomyosin in individual cells into a supracellular network which drives apical constriction and folding of mesoderm epithelium. Our previous studies show that the spot adherens junctions undergo myosin-dependent strengthening: increasing in packing density and size. This mechanosensitive response is essential for junction maintenance and mesoderm folding but its underlying molecular mechanism is unknown. We identified Drosophila casein kinase I gamma, Gilgamesh (Gish), as a potential mechanosensitive junction interactor and regulator that is essential for apical constriction. We found that Gish is recruited to the adherens junction puncta in response to myosin contraction. By tracking individual junction puncta, we identified two functions of Gish in myosin-dependent junction strengthening. First, Gish functions to promote the growth and merging of adherens junction puncta into larger clusters during apical constriction. Second, Gish functions to strengthen the association of spot junctions to the cell circumference to resist the pulling force from the contractile actomyosin. In Gish-deficient apically constricting mesoderm, small junction puncta form but are pulled to the apical top of the cell by contractile actomyosin, leading to gaps and foci of supracellular actomyosin network, and ultimately failed tissue folding. In addition, we found that actin nucleator Diaphanous is also recruited to the junction puncta during apical constriction, and Dia mutants resemble Gish mutants in the apical accumulation of junction puncta. These data suggest Gish may function through Diaphanous to regulate junction puncta and cell circumference association. Here, we show that Gish is a mechanosensitive junction interactor that strengthens adherens junctions through increasing junction clustering and enhancing junction-cell circumference association.

654T **Genome-wide expression profiling and phenotypic analysis of downstream targets identify the Fox transcription factor Jumeau as a master regulator of cardiac progenitor cell division** M. Rezaul Hasan[1,2,3], Andrew J. Kump[1,2,3], Evelyn C. Stepaniak[1,2,4], Manoj Panta[1,2], Kuncha Shashidhar[1,2,3], Rajnandani Katariya[1,2,3], Mofazzal K. Sabbir[1,2], Kristopher R. Schwab[1,2,3], Mark H. Inlow[2,5], Ye Chen[6], Shaad M. Ahmad[1,2,3] [1]Department of Biology, Indiana State University, [2]The Center for Genomic Advocacy, Indiana State University, [3]Rich and Robin Porter Cancer Research Center, Indiana State University, [4]School of Medicine, Indiana University, [5]Department of Mathematical Sciences, Indiana State University, [6]Department of Mathematics and Statistics, Northern Arizona University

Forkhead box (Fox) transcription factors (TFs) mediate multiple conserved cardiogenic processes in both mammals and *Drosophila*. Our prior work identified the roles of two *Drosophila* Fox genes, *jumeau* (*jumu*) and *Checkpoint suppressor 1-like* (*CHES-1-like*), in cardiac progenitor cell specification and division, and in the proper positioning of cardiac cell subtypes. Fox TF binding sites are also significantly enriched in the enhancers of genes expressed in the heart, suggesting that these genes may play a core regulatory role in one or more of these cardiogenic processes. We identified downstream targets of Jumu by comparing transcriptional expression profiles of flow cytometry-sorted mesodermal cells from wild-type embryos and embryos completely lacking the *jumu* gene and found that genes with functional annotation and ontological features suggesting roles in cell division were overrepresented among Jumu targets. Phenotypic analysis of a subset of these targets identified 21 *jumu*-regulated genes involved in cardiac progenitor cell division. One of these, *Retinal Homeobox* (*Rx*), was characterized in more detail and found to mediate all three known categories of cardiac progenitor cell division: symmetric, asymmetric, and cell divisions at an earlier developmental stage. Additional analysis also revealed a synergistic genetic interaction between *Rx* and *jumu* in mediating asymmetric cardiac progenitor cell divisions, a result which indicated that *Rx* and *jumu* function through the same genetic pathway. Chromatin immunoprecipitation data was also used to order the 21 *jumu*-activated cardiac progenitor cell division-mediating genes based on their likelihood of being directly regulated by Fox TF binding. Finally, we observed that many of these 21 genes and/or their orthologs exhibit genetic or physical interactions among themselves, indicating that Jumu is a master regulator that acts as a hub of a cardiac progenitor cell division-mediating network.

655T **Reconstructing the Fox transcription factor-regulated subnetwork that mediates specific cardiac progenitor cell divisions** M. Rezaul Hasan[1,2,3], Rajnandani Katariya[1,2,3], Kuncha Shashidhar[1,2,3], Mofazzal K. Sabbir[1,2], Andrew J. Kump[1,2,3], Manoj Panta[1,2], Kristopher R. Schwab[1,2,3], Mark H. Inlow[2,4], Shaad M. Ahmad[1,2,3] [1]Department of Biology, Indiana State University, [2]The Center for Genomic Advocacy, Indiana State University, [3]Rich and Robin Porter Cancer Research Center, Indiana State University, [4]Department of Mathematical Sciences, Indiana State University

Fox transcription factors mediate multiple cardiogenic processes in both mammals and *Drosophila*. The *Drosophila* Fox genes *jumeau* (*jumu*) and *Checkpoint suppressor 1-like* (*CHES-1-like*) mediate three distinct categories of cardiac progenitor cell division: asymmetric, symmetric, and cell division at an earlier stage. *jumu* also regulates the activity of the kinase Polo and the expression of the kinesin Nebbish (Neb) to bring about symmetric and earlier cardiac progenitor cell divisions in a *CHES-1-like*-independent process. Those observations raised two questions: whether other Fox TF-controlled genes mediating cardiac progenitor cell divisions are also regulated exclusively by *jumu* in a *polo*- and *neb*-like manner and whether such *jumu*-regulated genes mediate all three categories of cardiac progenitor cell division or a subset thereof. By comparing transcriptional expression profiles of wild-type, *jumu* loss-of-function, and *CHES-1-like* loss-of-function mesodermal cells, we identified multiple genes transcriptionally activated solely by *jumu*. Our phenotypic analysis of mutations of such exclusively *jumu*-regulated genes identified the anilin-encoding gene *scraps* (*scra*) as being required for only symmetric cardiac progenitor cell divisions and cell divisions at the earlier stage, while the citron kinase-encoding gene *sticky* (*sti*), the kinesin-encoding gene *pavarotti* (*pav*), and the Rho GTPase-encoding gene *tumbleweed* (*tum*) are required for all three categories of cardiac progenitor cell divisions. Synergistic genetic interactions between *scra*, *neb*, *jumu*, and *polo*, and the absence of such synergistic interactions between either *scra* and *CHES-1-like* or *neb* and *CHES-1-like*, demonstrate that *scra* and *neb* are integral components of a *jumu*- and *polo*-regulated subnetwork mediating a specific subset of cardiac progenitor cell divisions. Using further pairwise genetic interaction assays, we are attempting to assess whether *sti*, *pav*, and *tum* are additional components of this subnetwork. Subsequently, by utilizing qPCR and rescue assays, we intend to determine where each of these subnetwork components is positioned topologically relative to one another. Collectively, our results will illustrate how an individual regulator can utilize different combinations of downstream effectors to control distinct developmental processes.

656T **A bistable toggle switch regulated by Runt transcription factor governs the decision between two muscle cell fates** Jingjing Sun, Isaryhia Rodriguez, Angelike Stathopoulos California Institute of Technology

Robust cell fate decisions during embryogenesis rely on spatially patterned gene expression. One key question in developmental biology is how morphogen gradients and cis-regulatory mechanisms function to establish tissue compartments through precise gene expression boundaries. We previously discovered two distinct caudal mesoderm cell types arising simultaneously from the ventral, posterior (tail) region of the blastoderm embryo. These two neighboring cell types, marked by the expression of transcription factor genes FoxL1 in the anterior and HLH54F in the posterior, contain progenitor cells that contribute to the muscle linings of the developing hindgut and midgut, respectively. For FoxL1, we identified a 590bp enhancer capable of driving reporter expression, recapitulating its endogenous pattern. Through motif-based sequence analysis, we found that FoxL1 is controlled by Dichaete (D), a transcription factor expressed in the trunk region known to regulate the timing of pair-rule gene expression. In contrast, the previously identified 643bp HLH54F enhancer is bound by Forkhead (Fkh) as well as FoxL1. These findings suggested that HLH54F-positive cells relate to endoderm and that FoxL1 acts to restrict HLH54F expression. To focus on this specific cell fate decision, i.e., FoxL1 vs. HLH54F, we flattened the morphogen gradients by combining different sets of maternal mutations to obtain embryos expressing either of these two genes in the entire blastoderm. The flattened embryos that are HLH54F-positive also express huckebein (hkb) and tailless (tll) throughout but lose the expression of runt (run). The flattened embryos that are FoxL1-positive, on the contrary, maintain high expression of runt (run), consistent with our previous finding that Run promotes FoxL1-positive cell fate. These results indicate that different gene regulatory networks are at play in determining these two caudal mesoderm fates. In particular, we propose that Run levels establish a bistable toggle switch involving negative feedback loops to specify the boundary between FoxL1 and HLH54F gene expression domains. Specifically, high levels of Run support FoxL1 expression but limit HLH54F, and the mutual repression of HLH54F and FoxL1 functions to reinforce the cell fate decision. Furthermore, as Run is known to work collaboratively with other pair-rule factors, future experiments will aim to uncover the specific binding partners necessary to support its role in this binary cell fate decision.

657T **A novel protein Moat prevents ectopic epithelial folding by limiting Bazooka/Par3-dependent adherens junctions** Lingkun Gu[1], Rolin Sauceda[2], Jasneet Brar[1], Ferdos Fessahaye[1], Minsang Joo[3], Joan Lee[4], Jacqueline Nguyen[1], Marissa Teng[1] [1]UNLV, [2]Colossal Bioscience, [3]College of Osteopathic medicine, Touro University, [4]Case Western Reserve University

Contractile myosin and cell adhesion are critical players in the orchestration of tissue shape changes, working in tandem to drive complex morphogenetic outcomes. However, the mechanisms through which these elements are spatially and temporally patterned to support the diversity of tissue remodeling processes remain inadequately understood. A prime example of such remodeling is epithelial folding, which typically arises through apical constriction. This is well illustrated in the model organism *Drosophila*, particularly during the formation of the ventral furrow, where a gradient in myosin contractility within multiple cells drives the distinct shape of tissue folding. Although prior studies have shown that multicellular gradients in myosin activity are key to determining fold morphology, the role of multicellular patterning of adherens junction levels in influencing tissue folding remains unexplored.

In our study, we identified a novel *Drosophila* gene, named *moat*, that plays an essential role in enabling distinct apical constriction and folding behaviors within different regions of the ventral epithelium, specifically distinguishing between the folding ventral furrow and the non-folding ectodermal anterior midgut (ectoAMG). Our results show that Moat operates by downregulating polarity-dependent adherens junctions through the inhibition of Bazooka/Par3 protein clustering at the cortex. This downregulation of polarity-dependent adherens junctions is pivotal in establishing a pattern of myosin-dependent adherens junction distribution across the tissue, which in turn drives differential apical constriction in the ventral epithelial cells. In *moat* mutants, there is an aberrant increase in polarity-dependent adherens junction levels, which leads to inappropriate, ectopic apical constriction in cells with relatively low levels of contractile myosin. Consequently, this results in an uncharacteristic expansion of the infolding from the ventral furrow into the ectoAMG region, coupled with a flattened gradient of constriction within the ventral furrow. Our results demonstrate that tissue-scale distribution of adhesion levels patterns apical constriction and establishes morphogenetic boundaries. This work highlights the importance of adhesion patterning in modulating contractile dynamics to achieve distinct tissue shape changes, providing new insights into how multicellular architecture is regulated during development.

658F **Gαq Homeostasis is Required for Organ Size Regulation and Proper Timing of Larval to Pupal Metamorphosis in *Drosophila melanogaster*** Maria Unger[1], Vijay Velagala[2], Dharsan Soundarrajan[2], David Gazzo[2], Nilay Kumar[2], Marycruz Flores Flores[2], Jinwen Liu[2], Jun Li[2], Jeremiah Zartman[2] [1]Biomolecular Engineering, University of Notre Dame, [2]University of Notre Dame

The G protein alpha subunit, Gαq, plays a critical role in mediating signal transduction downstream of numerous G-protein-coupled receptors (GPCRs), influencing various physiological processes essential for health and development. For example, mutations in GNAQ, the Gαq human ortholog, have been associated with significant pathologies such as Weber-Sturges syndrome and uveal melanoma. Despite these associations, the downstream effectors of Gαq, and its precise functional roles in organogenesis and other cellular processes remain poorly understood. Through RNA-sequencing (RNA-seq), phenotypic functional analysis and immunohistochemistry, we discovered that overexpression of *Gαq* in *Drosophila* imaginal wing discs elicits a similar response triggered by wounding and infection. This includes transcriptional upregulation of JAK/STAT and Toll signaling pathways and the secretion of *Drosophila* insulin-like peptide 8 (Dilp8), which induces a developmental delay. Through functional validation, we confirmed that Gαq mediates Dilp8 signaling via inositol 1,4,5-triphosphate Receptor (IP3R)-dependent calcium (Ca^{2+}) release and that developmental delay caused by *Gαq* overexpression can be rescued by inhibiting Dilp8. In summary, Gαq homeostasis is essential for achieving the optimal organ size by regulating the timing of pupal metamorphosis via Dilp8 release. This study elucidates the connection between Gαq-mediated calcium signaling and downstream Dilp8-signaling and highlights how calcium dynamics contribute to the regulation of key pathways involved in wound response and tissue regeneration.

659F **The Role of Dsx in Sexually Dimorphic Development of Somatic Gonads** Jiaxin Li[1], Mark Van Doren[2] [1]Department of Biology, Johns Hopkins University, [2]Johns Hopkins University

Male or female? This is not just a simple guess with a 50/50 chance to get the right answer, but an important and widespread biological decision made by diverse mechanisms among different organisms, which further mediate sex-specific development throughout life. In Drosophila, a critical component of the sex determination pathway is *doublesex (dsx)*, which undergoes alternative splicing to generate male/female isoform: Dsx^M/Dsx^F. However, the downstream of Dsx is not well studied yet.

A fascinating example of sexually-dimorphic development in Drosophila gonads is that, by the end of embryogenesis, male gonads have already established the stem cell niche (hub) and the somatic stem cells (Cyst Stem cells or CySCs). Previously we showed that regardless of sex genotype, *dsx* null mutants initiate the male pattern of development in embryonic gonads. Screening with different Gal4 lines, we found that ectopic expression of tra^F with twist-Gal4 and abd-A-Gal4, but not other mesoderm or somatic gonadal precursor (SGP) drivers, can establish full sex transformation. Only abd-A-Gal4 and twist-Gal4 can induce expression in all SGPs during embryo-to-larvae transition and maintain their expression in the niche during larval stage. Since Dsx's expression is restricted to SGPs during the embryo-to-larvae transition, we hypothesize that correct sex-specific development is a cell-autonomous decision that requires all SGPs to have the same sex identities.

Moreover, manipulating putative Dsx target genes *abd-A* or *Abd-B*, as well as the mesoderm gene *twist,* show sex-dimorphic phenotypes in male and female gonads, especially in the stem cell niche, suggesting that Dsx is potent regulator that controls upstream developmental genes to establish sex-specific niches, which further mediate sex-specific development. We are currently working on profiling L3 gonads with altered Dsx activity by single nucleus RNA sequencing (snRNA-seq) and ATAC seq, which will further contribute to our understanding of the logic by which Dsx acts to control dimorphic developmental outcomes in different tissues in the context of multiple highly integrated networks.

660F **Multiple isoforms of RNA-binding protein Bruno1 are required during indirect flight muscle development in Drosophila** Jenna A.M. DeCata[1], Elena Nikonova[2], Sienna Ficken[1], Thomas Harr[2], Danei Smith[1], Maria Spletter[1] [1]School of Science and Engineering, University of Missouri-Kansas City, [2]Ludwig-Maximilians-Universitat Munchen

During development, indirect flight muscles (IFMs) of *Drosophila* undergo a switch in fiber-type specific alternative splicing that is essential for flight behavior. This switch is regulated by RNA-binding proteins (RBPs), especially Bruno1 (Bru1). Bru1 is a conserved member of the CELF family, which are important regulators of developmental alternative splicing in vertebrate striated muscle. CELF activity is misregulated in patients with Myotonic Dystrophy Type I, notably resulting in a reversion to embryonic splicing patterns and indicating that CELF function is critical to muscle development and function. In flies, Bru1 is known to play a role in translation repression during embryo formation, but recent work identified Bru1 as a muscle fiber-type specific splicing factor which is necessary for a developmental transition to IFM-specific splice isoforms. Bru1 promotes early cytoskeletal rearrangements enabling myofibrillogenesis and during later stages it promotes maturation of the sarcomeres and regulates myosin contractility. Bru1 is alternatively spliced to produce at least 6 protein isoforms, but isoform-specific CELF functions are not reported in flies or vertebrates. Here we test if Bru1 isoforms play distinct roles in myogenesis. We show that an isoform-specific CRISPR mutant affecting long Bru1 isoforms develops normally but has a hypercontraction phenotype, which differs from severe phenotypes in pan-isoform Bru1 mutants. Using Gal4-UAS to control when and where specific Bru1 isoforms are expressed, we test the function of long (isoform B), middle (isoform A), and short (isoform D) isoforms. Our results show distinct localization patterns for Bru1 isoforms in myogenesis. Using Him-Gal4 and Fln-Gal4 to drive early and late overexpression, respectively, we show that overexpression of Bru1-isoB results in a strong muscle detachment phenotype, while Bru1-isoA overexpression disrupted sarcomere structure. This phenotype is dosage dependent, and we use temperature shifting as well as alternate UAS promoters to modulate Bru1 expression levels. Bru1-isoD overexpression resulted in only minor muscle defects. We further tested isoform-specific rescue ability and find that a combination of Bru1-isoA and Bru1-isoD are required to rescue IFM development. These differences suggest a requirement for distinct Bru1 isoforms during muscle development and are the first demonstration of CELF-family isoform-specific function in myogenesis.

661F **Fox transcription factor-mediated morphogenesis of the alary muscles associated with the Drosophila heart.** Kuncha Shashidhar, Rajnandani Katariya, Rezaul Hasan, Mofazzal K Sabbir, Shaad M Ahmad Biology, Indiana State University

Eight Forkhead box (Fox) transcription factors are required for proper cardiogenesis in mammals, with mutations in four Fox genes linked to human congenital heart diseases (CHDs). Our previous work identified the conserved roles of two Drosophila Fox genes, *jumeau* (*jumu*) and *Checkpoint suppressor 1-like* (*CHES-1-like*), in cardiac progenitor cell specification, division, differentiation, and positioning. Here, we explore additional roles for *jumu* and *CHES-1-like* in the development of pericardial ligaments called Alary Muscles (AMs) which anchor the heart to the exoskeleton, provide structural support, and stabilize heart position. In *jumu* or *CHES-1-like* loss-of-function mutants, one or more of the 14 AMs per embryo exhibit significant morphological defects, being deformed, truncated, missing, or incorrectly positioned or attached. Each AM forms as a syncytial myotube through the fusion of a unique Founder Cell (FC) with Fusion Competent Myoblasts (FCMs), eventually attaching to the extracellular matrix of seven up-expressing Pericardial Cells (Svp-PCs). Hence, one or more of the following hypotheses could explain the defects in AM morophogenesis: incorrect specification of AM FCs, defective myoblast fusion, errors in myotube elongation, unexpected fusion between distinct myotubes, and incorrect muscle attachment.

We are conducting a series of experiments to determine which of these hypotheses are correct. We are assessing relevant AM FC-specific transcription factor expression to determine if FC specification is defective. Quantitation and comparison of myonuclei counts in AM myotubes are being used to assess potential myoblast fusion defects. Live imaging and tropomyosin staining will be utilized to assess potential errors in myotube elongation or abnormal fusion between two myotubes. Muscle attachment defects could be due to myotubes failing to associate with Svp-PCs or there being an incorrect number of attachment sites (Svp-PCs) in the Fox mutants. The former will be detected via immunostaining and imaging, and independent alteration of Svp-PC numbers will be used to assess if the Fox mutant AM defects are phenocopied.

Pericardial disorders associated with pericardial ligaments often lead to sudden cardiac death in humans. Improving our understanding of Fox-mediated AM development in Drosophila may thus lead to advances in diagnostics and treatment of pericardial disorders and CHDs.

662F **Exploring the Role of Signaling Molecules During Posterior Migration of the *Drosophila* Salivary Gland** Ashleigh M Shoemaker[1], Dan Peng[2], Liliana Wang[1], Patrick Cahan[2], Deborah J Andrew[1] [1]Cell Biology, Johns Hopkins University School of Medicine, [2]Biomedical Engineering, Johns Hopkins University School of Medicine

The fruit fly (*Drosophila melanogaster*) has long been utilized for understanding complex signaling transduction pathways. Our laboratory employs the embryonic salivary gland (SG) as a model of simple epithelial tube formation -- a process that involves cell invagination and directed cell migration. In particular, this project aims to investigate the posterior migration of the SG, which occurs after the distal tip of the invaginating SG contacts the visceral mesoderm (VM). This interaction -- one that requires integrins -- prompts the SG to reorient from an approximately dorsally directed to a posteriorly directed trajectory. Whereas the tissue substrates that mediate posterior SG migration have been well-described by our laboratory, questions regarding the signaling interactions that contribute to this process remain. We hypothesize that using our published scRNA-seq data, we will discover ligand and receptor pair(s) that work together to control SG migration along the circular visceral mesoderm (cVM). Using manual and computational analysis of our wild-type whole embryo scRNA-seq data in comparison to a web-based repository of intra- and intercellular signaling molecules in *Drosophila*, we identified candidate molecules expressed during the initial stages of SG migration. Our work revealed 48 unique signaling receptors expressed in the SG, with many dedicated to GPCR or Wnt-related pathways. Through phenotypic assessments of an initial pool of null mutants, we detected at least two receptors that prevent or significantly alter SG migration, *off-track* (*otk*) and *Calcium-independent receptor for α-latrotoxin* (*Cirl*). Both of these receptors were initially studied in relation to the nervous system, and have not thus far been identified as players in SG organogenesis. With this, we show how scRNA-seq datasets can be utilized to predict ligand-receptor interactions mediating morphogenetic processes from gene expression in their corresponding cell-specific clusters. Further analysis of candidate ligand-receptor pairs will allow us to continue to discover new players, their tissue sources, and the consequences of their loss on SG formation.

663F **More than just a phenotypic marker, *Drop* is involved with the formation of the salivary gland.** Matthew Elliott[1], Deborah Andrew[2] [1]Cell Biology, Johns Hopkins University, [2]Cell Biology, Johns Hopkins School of Medicine

Epithelial tube development is essential in the formation of many vital organs and defects in tube morphogenesis is linked to several developmental disorders. To study epithelial tube development, our lab utilizes the salivary gland (SG) of *Drosophila melanogaster.* We have discovered several transcription factors that control development and specialization of the SG during embryogenesis. Recently, single cell RNA sequencing (scRNA-seq) data from our lab revealed expression of *Dr*op (*Dr*) mRNA in a subset of cells of the SG of *D. melanogaster* during the earliest stages of its development. *Dr* encodes for a NK-like homeodomain transcription factor that is homologous to the human Msx1 and Msx2 proteins. *Dr* is best known for its dominant gain-of-function small eye phenotype that is commonly used as an easy to identify genetic marker in crosses of the 3rd chromosome. Previous work has revealed that *Dr* function is required for specification of myoblasts and neuroblasts, development of muscle and neuronal cells, regulation of glucose metabolism, and patterning of the neuroectoderm and wing disc. *Dr*'s role in the SG has not yet been described. Our preliminary findings from immunostained embryos from a *Dr* insertion line revealed a change in SG morphology consistent with abnormal cell invagination. Furthermore, our scRNA-seq data showed *Dr* mRNA expression in the subset of cells that internalize first during SG morphogenesis. We hypothesize *Dr* may function to determine which cells in the secretory placode are the first to internalize. To test this idea, we have developed GFP tagged and untagged Gal4/UAS-expression constructs to drive persistent ectopic expression of *Dr* in the SG, we have developed CRISPR-Cas9 *Dr* knock-outs to determine the effects of null mutations of *Dr* on the formation of the SG, and we've generated an antibody directed against *Dr*. This newly generated Dr antibody has allowed us to demonstrate that Dr protein (like *Dr* mRNA) is expressed in SG cells that internalize first and will allow us to further characterize our CRISPR-Cas9 *Dr* mutants. Moreover, our findings may shed light on the role of the human orthologs Msx1 and Msx2 in early developmental processes and in cancer.

664F **The Influence of the Tok Protease and Subsequent Slit Fragments on Heart Development and Function in *Drosophila*** Sahara C Harrington[1], Riley Kellermeyer[2], Ria Anand[1], Jonathan Taasan[3], Thomas Kidd[4] [1]Cell and Molecular Biology, University of Nevada, Reno, [2]University of Minnesota, [3]University of Colorado, Boulder, [4]Biology, University of Nevada, Reno

During development, cardiac precursor cells must properly migrate and align to produce a functioning heart. Axon guidance genes like Slit-Roundabout (Robo) are involved in cardiac development, causing functional and phenotypic defects when disrupted. *Drosophila melanogaster* are an excellent model for studying cardiac defects due to their conserved, non-redundant genetic pathways, parallel cardiac phenotypes, and simple genetic tools. While Slit-FL is well characterized, recent findings reveal that proteolytic cleavage by Tolkin (Tok), produces an N-terminal fragment (Slit-N) and a C-terminal fragment (Slit-C) with distinct functions in the CNS, and preliminary data shows this is also true in the cardiac tube. Previous studies show that Slit/Robo signaling contributes to cardiac system formation, likely through interactions with collagens, but the underlying mechanisms remain unclear. Given the diverse response of axons to Slit-receptor signaling, we hypothesize that Tok protease cleavage of Slit produces fragments that enhance cardiac cell motility and adhesion, ensuring proper heart development and function in *Drosophila melanogaster*. Despite 30 years of researching Slit-Robo signaling in heart development, the genetic tools necessary to investigate the role of these fragments in cardiac cell adhesion and migration were lacking. Most studies on heart development through Slit signaling do not report the specific Slit isoform, making it crucial to clarify the function of each fragment. With access to advanced imaging and computing technologies at the University of Nevada, Reno, and over 20 years of dedicated research by the Kidd Laboratory on the Slit network, we are uniquely positioned to undertake this project. Preliminary data demonstrates different roles for each Slit fragment and Tok in cardiac development. Using genetic manipulations, molecular experiments, and functionality assays, we show that the fragments of proteolytically cleaved Slit uniquely contribute to cardiac development compared to their full-length (Slit-FL) counterpart, possibly through collagen interactions. We will use novel CRISPR Slit alleles generated by our lab to elucidate molecular mechanisms and fragment functions and are developing a novel machine learning algorithm to detect cardiac functionality defects. The proposed research investigates the interaction between Slit and Tok, providing insights into heart development across species, including primitive heart tube and organ formation. Our study elucidates the molecular and biological mechanisms of Tok and Slit fragments, suggesting they have role in the coordinated movement and adhesion of cardiac cells necessary for heart valve formation in humans.

665F **Shining Light on Calcium-Mediated Morphogenesis: Forward Engineering Organ Development with Optogenetics** Mayesha Mim[1], Jeremiah Zartman[2] [1]Chemical and Biomolecular engineering, University of Notre Dame, [2]Chemical and Biomolecular Engineering, University of Notre Dame

Calcium (Ca^{2+}) serves as a key second messenger in a multiscale coordination for cells to communicate and coordinate processes across tissues. Ca^{2+}-selective channels regulate the influx of Ca^{2+} into the cytosol upon activation, but there is a gap in our understanding of these mechanisms due to limited direct control over Ca^{2+}-mediated processes. Here, we used an optogenetic channelrhodopsin, CsChrimson, to investigate how Ca^{2+} signaling impacts epithelial growth and morphogenesis in Drosophila melanogaster. We observed that controlling Ca^{2+} levels impacts both cell proliferation and cell death. We identified the optimal biphasic cytosolic Ca^{2+} response level for final organ size adjusting light intensity and activation cycles. Our results show that CsChrimson modulates cytosolic Ca^{2+} dynamics, influencing several downstream proteins and growth-related pathways. Prolonged light activation (>6 hours) led to increased cell death in wing imaginal discs and severe morphological changes in adult wings, correlating with the intensity and duration of light exposure. In sum, these findings reveal the potential for using optogenetics to precisely control organ development, offering new insights for phenotypic drug screening and therapeutic innovations in human disease treatments.

666F **Eicosanoid signaling in *Drosophila melanogaster*** Daiki Fujinaga, Naoki Yamanaka Entomology, University of California, Riverside

20-carbon fatty acid-derived eicosanoids are versatile signaling oxylipins in mammals. In particular, a group of eicosanoids termed prostanoids are involved in multiple physiological processes, such as reproduction and immune responses. Although some eicosanoids such as prostaglandin E2 (PGE2) have been detected in some insect species, molecular mechanisms of eicosanoid synthesis and signal transduction in insects have been poorly investigated. Our phylogenetic analysis indicated that, in clear contrast to the presence of numerous receptors for oxylipins and other lipid mediators in humans, the *Drosophila* genome only possesses a single ortholog of such receptors, which is homologous to human prostanoid receptors. This G protein-coupled receptor, named Prostaglandin Receptor or PGR, is activated by PGE2 and its isomers in *Drosophila* S2 cells. *PGR* mutant flies die as pharate adults with insufficient tracheal development, which can be rescued by supplying high oxygen. Consistent with this, through a comprehensive mutagenesis approach, we identified a *Drosophila* PGE synthase whose mutants show similar pharate adult lethality with hypoxia responses. *Drosophila* thus has a highly simplified eicosanoid signaling pathway as compared to humans, and it may provide an ideal model system for investigating evolutionarily conserved aspects of eicosanoid signaling in the animal kingdom.

667F **From neurogenesis to oogenesis: Investigating Inscuteable's role in *Drosophila* oocytes** Sahel Ghasemzadeh, Dan T Bergstralh Biological Sciences, University of Missouri Columbia

In fruit flies a single germline stem cell undergoes division to form a cluster of sixteen interconnected cells. Among these cells one transforms into the oocyte while the remaining fifteen differentiate into nurse cells, for supplying nutrients and support the oocyte's growth and development. The process of oocyte specification involves careful control of gene expression. This control is essential for determining which cell becomes the oocyte. When this process fails, it can lead to problems, such as having multiple oocytes or no oocyte at all.

Recent advancements in single-cell RNA sequencing allow us to analyze gene expression across cell types. This approach revealed expression of **Inscuteable** mRNA in the fly ovary. This finding is unexpected because Inscuteable, which has long been studied for its role in controlling asymmetric cell division in the developing nervous system, is almost unknown outside the CNS. Using a combination of immunostaining and mRNA expression analysis, we determined that Inscuteable is expressed in the oocyte during early stages of egg chamber development. We are now using a combination of classic *Drosophila* genetic methods and advanced imaging techniques to clarify Insc›s role in the oocyte.

668F **Unraveling the interplay of SNARE proteins in wing morphogenesis** Mikaela Follmer[1], Samantha Ramirez[2], Jasmynn Calderon[2], Emily Bates[3] [1]Developmental Biology, University of Colorado - Anschutz Medical Campus, [2]University of Colorado Denver, [3]University of Colorado - Anschutz Medical Campus

How do cells regulate the timing and strength of developmental signals to pattern complex structures? The cellular mechanism controlling release of developmental morphogens at the right time and place is unknown. Evidence from my lab shows that depolarization induces release of the developmental signal Decapentaplegic (Dpp) suggesting that the mechanism of Dpp release relies on electrical activity. We discovered that the larval wing discs and pupal wings have endogenous calcium oscillations, indicative of electrical activity. We found that inhibition of endoplasmic reticulum calcium regulators Stim and SERCA abolishes calcium oscillations and reduces Dpp release and downstream signaling. Due to Dpp's reliance on intracellular calcium activity for release, I looked to neurons for insights into the machinery controlling Dpp release. In neurons, calcium stimulates the SNARE complex to induce vesicle fusion and neurotransmitter release. Five SNARE proteins required for neurotransmitter release are also expressed in the L3 wing disc, Synaptobrevin (Syb), Synaptotagmin (Syt), Snap24, Snap25, and Syntaxin1A (Syx1A). Knockdown of each SNARE protein with RNAi causes wing vein patterning defects ranging from vein bifurcation and missing anterior cross veins to ectopic veins and wing blistering. These defects phenocopy disruption of the Dpp signaling pathway. To investigate how the SNAREs affect Dpp signaling, I measured phosphorylation of MAD (pMad) as a readout of Dpp signaling in wing discs with wing-specific knockdown of Syx1A. I found Syx1A knockdown increases pMad in wing discs, meaning that Syx1A is a negative regulator of Dpp. To determine which wing disc cells required Syx1A for Dpp signaling, I knocked down Syx1A in only Dpp-producing cells. Knocking down Syx1A in the Dpp-producing cells (*Dpp-Gal4*) caused a significant increase in pMad signal, the same result as whole wing knockdown. Further validating that Syx1A acts as a brake to attenuate Dpp release from Dpp producing cells. In contrast, knocking down Syx1A in Dpp receiving cells using the clonal Flp-FRT system resulted in a significant decrease in pMad signal – a Dpp pathway loss-of-function phenotype. Thus, Syx1A has a cell autonomous function in Dpp receiving cells where it promotes reception of the Dpp signal. In summary, our results indicate that the SNARE protein Syx1A controls the strength of the Dpp signal through distinct mechanisms in Dpp-producing and Dpp-responding cells.

669F **Endoplasmic Reticulum Calcium Homeostasis is required for Dpp release and wing patterning in *Drosophila*** Emily Bates, Mikaela Follmer, Laura George Pediatrics, University of Colorado Anschutz Medical Campus

The temporal dynamics of morphogen presentation impacts transcriptional responses and tissue patterning. However, the mechanisms controlling morphogen release are far from clear. We found that inwardly rectifying potassium (Irk) channels regulate endogenous transient increases in intracellular calcium and bone morphogenetic protein (BMP/Dpp) release for *Drosophila* wing development. Irk channel inhibition reduces BMP/Dpp signaling, and ultimately disrupts wing morphology. Ion channels impact development of several tissues and organisms in which BMP signaling is essential. In neurons and pancreatic beta cells, Irk channels modulate membrane potential to affect intracellular Ca++ to control secretion of neurotransmitters and insulin. Based on Irk activity in neurons, we hypothesized that electrical activity controls endoplasmic reticulum (ER) Ca++ release into the cytoplasm to regulate the release of BMP. To test this hypothesis, we reduced expression of four proteins that control ER calcium, Stromal interaction molecule 1 (Stim), Calcium release-activated calcium channel protein 1 (Orai), SarcoEndoplasmic Reticulum Calcium ATPase (SERCA), small conductance calcium-activated potassium channel (SK), and Bestrophin 2 (Best2) using RNAi and documented wing phenotypes. We use live imaging to study calcium and Dpp release within pupal wings and larval wing discs. Additionally, we employed immunohistochemistry to characterize Small Mothers Against Decapentaplegic (SMAD) phosphorylation downstream of the BMP/Dpp pathway following RNAi knockdown. We found that reduced Stim and SERCA function decreases amplitude and frequency of endogenous calcium transients in the wing disc and reduced BMP/Dpp release. Our results suggest control of ER calcium homeostasis is required for BMP/Dpp release for *Drosophila* wing development.

670F **Tissue-intrinsic signaling affects spermatogonial niche formation** Ariel M Harrington, Lauren Anllo Biology, East Carolina University

Stem cells play an important role in tissue maintenance and self-renewal. A cellular microenvironment, the niche, is required to maintain stem cells. Understanding how a niche forms and functions is key to stem cell biology. The *Drosophila* testis niche provides an optimal model to study niche formation in an organism that is genetically modifiable and enables complete *in vivo* visualization of niche formation with subcellular resolution. The testis niche forms at the anterior of the embryonic gonad. At this stage of development, the gonad is a spherical arrangement of germ cells encysted by somatic cells. Directly opposite the niche, at the posterior, is a group of somatic cells called male specific gonadal precursor cells (msSGPs) that coalesce with the gonad prior to niche formation and eventually develop into the terminal epithelial (TE) cells of the larval gonad. We have ablated these msSGPs using two different genetic means and found that the niche has disrupted morphology or clusters away from the gonad anterior, suggesting that msSGPs are required to form a compact anterior niche. We also found that without msSGPs, the disrupted niches that form fail to polarize the F-actin cytoskeleton, which previous work identified as required to establish proper niche morphology (Anllo & DiNardo, 2022; Warder et al., 2024). These results, in combination with the specific positioning of msSGPs at the posterior, suggest a possible repulsive cue originating in these cells that serves to localize the compacted niche at the gonad anterior. We have turned to recent scRNA sequencing data (Mahadevaraju et al., 2022) to identify candidate signaling genes expressed in TE cells. Our data confirms that Nord, a secretory protein that modulates diffuse signals, is expressed in msSGPs and plays a role in niche compartmentalization at the anterior. Our goals include defining the mechanisms by which msSGPs, and msSGP expression of *nord*, enable proper assembly of the testis niche using tissue-specific ablation methods and two *nord* mutant alleles. As concepts in stem cell niche biology have repeatedly translated from the *Drosophila* testis to other systems, we aim to uncover novel concepts required to establish a stem cell microenvironment.

671F **Tbx1 ortholog *org1* is required to establish testis stem cell niche identity** Patrick Hofe[1], Tynan Gardner[2], Kirklan Naumuk[3], Stephen DiNardo[2], Lauren Anllo[3] [1]Biology, East Carolina University, [2]University of Pennsylvania Perelman School of Medicine, [3]East Carolina University

Tissue homeostasis is mediated by stem cells and their progeny, as tissues rely on stem cells to maintain diversity of cell types. To carry out this function, stem cells require signals from a cellular microenvironment known as the stem cell niche. The niche regulates identity, location, and division of stem cells. The *Drosophila* testis provides an excellent model to study stem cells and their local niche interactions, as concepts unearthed in this model have repeatedly translated to other model systems. We utilize the *Drosophila* embryonic gonad to study the initial establishment of this niche during development. During embryogenesis, germ cells are encysted by somatic gonadal precursor cells (SGPs) at the location of the future gonad, where the gonad coalesces into a sphere. At this point, the SGPs that will become niche cells (pro-niche cells) are already specified and reside in the anterior half of the gonad. These cells must migrate to the gonad anterior to establish the niche in response to extrinsic tissue signals from visceral muscle that surrounds the gut. The assembled niche is later anchored at the gonad anterior and surrounded radially by germline and somatic stem cells. Our previous work identified a role for the transcription factor Islet in assembly of this niche downstream of visceral muscle signals (Anllo & DiNardo, 2022). Because *islet* expression is known to be regulated by the T-box transcription factor Org1 to specify embryonic muscle (Boukhatmi et. al., 2014), we hypothesized that *org1* might also play a role in niche establishment and regulation of niche *islet* expression. Upon examining *org1* mutants, we noticed that they had reduced Islet accumulation, lacked niche adhesion marker Fas3, had fewer niche cells, and exhibited various functional deficits. Additionally, we found that overexpressing *org1* in all SGPs creates more niche cells, suggesting that *org1* is both necessary and sufficient for niche cell identity. We show that Org1 accumulates in response to visceral muscle signals, and that *islet* overexpression can rescue niche identity in *org1* mutants. These data unveil Org1 as a novel factor that regulates niche cell identity upstream of *islet* in the *Drosophila* testis stem cell niche. Our current and future work focuses on identifying transcriptional targets of Org1 and how these mediate niche identity. This addresses mechanisms necessary to induce niche cell specification to create a functional niche.

672S **Morphogen scale invariance does not explain the environmental robustness of *Drosophila* wing pattern** Bhagyashree A Ghag[1], Alexander Shingleton[2] [1]Biological sciences, University of Illinois Chicago, [2]Biological sciences, University of Illinois at Chicago

The shape of an organism is determined by the spatial arrangement of its morphological traits, referred to as 'pattern'. To preserve function, pattern must be maintained as body size varies with genotype and the environment. How the pattern and size are developmentally coordinated is poorly understood. Canonically, pattern formation is orchestrated by diffusible signaling molecules known as morphogens. These morphogens are secreted to form a gradient across developing tissue. Cells at different positions along the gradient are exposed to different concentrations of the morphogen, which determines their fate. Previous data demonstrate that morphogen gradients scale proportionally to the size of the growing tissue, a phenomenon called dynamic scale invariance, which is hypothesized to account for the maintenance of pattern regardless of final tissue size. This hypothesis, however, has not been tested, when final tissue size varies with environmental conditions. In this study, we use *Drosophila melanogaster* wing discs as a model to explore the effect of environmental variation on morphogen scaling, focusing on the Decapentaplegic (Dpp) morphogen gradient, which controls the position of the longitudinal veins in the wing. We show that, consistent with previous studies, the Dpp signaling gradient (as assayed by phosphorylated mothers against Dpp, pMad) is dynamically scale invariant under standard laboratory conditions (25˚C, 21% O_2), throughout ontogeny. However, at low oxygen (10 kPa O_2, 25˚C) and low temperature (17˚C, 21 kPa O_2) Dpp signaling gradient is not dynamically scale invariant but becomes disproportionally narrower as the disc grows. Despite this underscaling, the pattern of longitudinal veins is maintained across environmental conditions that almost double adult wing size. These data indicate that dynamic scale invariance is not an explanation for the maintenance of pattern across a range of wing sizes and suggest another uncharacterized mechanism must be involved.

673S **STIL is specifically expressed and required in the *Drosophila* female germline** charli L Wingfield[1,2], Leif Benner[1], Brian Oliver[2], Leah Rosin[1] [1]NICHD, NIH, [2]NIDDK, NIH

Female germ cells go through a dramatic differentiation process to give rise to a viable oocyte. Numerous RNAs and proteins are required during this process, however, the transcriptional control of these programs are less understood. The gene, *stand still* (*stil*), encodes a euchromatin binding protein that is required for female germline development in *Drosophila*. *stil* has been shown to be bound by and transcriptionally responsive to the female germ cell-specific transcription factor OVO. Therefore, we are interested in determining the function of STIL and interaction with OVO that help drive female germ cell differentiation. We first created and tested novel *stil* loss-of-function alleles and endogenously tagged *stil* with a 3xFLAG-HA and GFP peptide with CRISPR/Cas9. We found that adult female germ cells mutant for *stil* do not survive and that STIL localizes to germ cell nuclei in all stages of oogenesis. We also found that STIL is maternally deposited and localizes to the nucleus of embryonic stage 4 pole cells. Following the dynamics of STIL localization throughout embryonic development, we found that maternal STIL is degraded around stage 8 but becomes zygotically expressed in the germline around stage 12. Using our endogenously GFP tagged *stil* allele, we performed ChIP-seq and saw enrichment over the *stil* locus, *CTCF*, and the histone locus body, among others. We also saw that the ChIP peak maximums align with nucleosome bound euchromatin based on H3K27ac ChIP. Altogether, these tools will allow us to determine where STIL is binding genome-wide and what genes are transcriptionally responsive to STIL through ChIP- and RNA-seq. We are also able to Co-IP to see what other proteins STIL may interact with further allowing us to understand the role of STIL in female germ cell development.

674S **Defining the function of extracellular matrix proteins in the development of Drosophila genital structures** Daniel Ruiz, Jaysonn Garcia, Cindy Nguyen, Nahlat Zein, Ben J Vincent Biological Sciences, California State University – Los Angeles

In development, living organisms undergo a myriad of changes at the cellular level. These changes are orchestrated by regulatory genes that activate effector proteins in particular cell populations. Defining these regulatory connections is essential for understanding how animal body parts develop and evolve. Our research focuses primarily on the genes that influence the shape and size of genital structures in Drosophila melanogaster, as these structures are developmentally tractable and evolve rapidly between closely related species. Previous work has found that the extracellular matrix (ECM) protein dumpy is necessary for proper development of the male genitalia, but the precise role of other ECM proteins in this process remains unknown. To test the function of these proteins, we measured their gene expression patterns during genital development using in situ hybridization. We found that multiple ECM proteins are expressed in precise patterns during genital formation, including some that serve as markers for particular genital structures. For example, piopio appears localized to the developing clasper, while dusky-like is restricted to the developing phallus and surrounding bristle cells. We are following up on these results in two ways. First, we are using hybridization chain reaction and confocal microscopy to measure the expression patterns of ECM genes at high resolution in 3-dimensional space. This approach also allows for multiplexing to determine whether ECM genes are co-expressed within developing tissues. Second, we are using RNAi to knock down ECM genes during pupal development in order to measure phenotypic changes in the size and shape of genital structures. This approach will illuminate whether additional ECM components are necessary for genital development, and may indicate functional redundancies between different ECM genes. Together, these experiments will define the role of the multicomponent extracellular matrix in the development of complex animal body parts, which may prove useful in determining how those structures evolve.

675S **Transmembrane Receptor Plexin A and its Known Ligands Semaphorin 1b and Semaphorin 5c are Required for Proper Tube Elongation in *Drosophila melanogaster*** Haley R Parrett, Celeste A Berg Department of Genome Sciences, University of Washington

A process of tube formation occurs within *Drosophila melanogaster* oogenesis and is known as “wrapping.” From a flat sheet of cells, a patch of cells constricts their apical (inner) surfaces and expands their outer surfaces, bending the patch up and out of the plane until the edges make contact with one another, thus forming a tube. The tube cells then intercalate and migrate to elongate and narrow the tube. This conserved process closely mirrors the neurulation of the ectoderm during vertebrate embryogenesis. Tubulogenesis of the dorsal appendage (DA) eggshell structures during *Drosophila melanogaster* oogenesis serves as a model system for understanding how signaling pathways and patterning can influence cell motility and morphological changes. Previous work in the Berg lab identified a novel family of growth factors, called Chitinase-Like Proteins (CLPs), as regulating tube wrapping and elongation [Zimmerman et al. 2017; Sustar et al. 2023]. A genetic interaction screen [Espinoza and Berg 2020] led to the hypothesis that Plexin receptors act as potential interactors with the CLP growth factors. The Plexin-Semaphorin signaling pathways are well known for their roles in regulating the motility of neuronal growth cones through the transmission of repulsive growth signals during cell migration. Recent discoveries have also shown that Plexin-Semaphorin signaling regulates other cell migratory processes and tissue development. Through Gal4-driven RNAi knockdown and over-expression of the known elements of Plexin-Semaphorin signaling in the follicular cells of the developing fruit fly egg chamber, we have found clear morphological disruptions of DA formation specifically related to the dysregulation of Plexin A and two of its known ligands, Semaphorin 1b and Semaphorin 5c.

676S **Control of cell division orientation by patterned cell-surface receptors during axis elongation** Chloe A Kuebler, Adam C Paré Biological Sciences, University of Arkansas

Cell divisions during animal development play a key role in determining tissue architecture. Directionally oriented cell division is a conserved phenomenon of epithelial remodeling that elongates tissues and separates functionally distinct cell populations. Improper cell division orientation contributes to tissue architecture defects, cancer invasion, and cell fate misspecification. An excellent model for studying oriented cell divisions is germband extension of the early *Drosophila* embryo, in which hundreds of epithelial cells divide in a coordinated manner along the head-to-tail axis. The elongating germband comprises two mechanically distinct tissues: the lateral neuroectoderm and the ventral mesectoderm. Tissue-level mechanical forces generated by contractile actomyosin can influence the direction of cell division during *Drosophila* germband extension. Previous studies have demonstrated that tension along the head-to-tail axis is required to orient cell divisions in the ventral mesectoderm, and the patterning genes that define this same axis contribute to division orientation in the lateral neuroectoderm. However, the nature of the molecular cues that direct cell division orientation is unknown. The head-to-tail patterning system regulates the striped expression of leucine-rich-repeat encoding genes during germband extension. The overlapping striped expression of these genes is required to establish polarized actomyosin at the onset of germband extension, directly proceeding oriented cell divisions. Therefore, we tested whether these same striped cues are also responsible for directing cell division angle. To address this question, we tracked cell division angle in the lateral neuroectoderm and ventral mesectoderm during the germband extension in live *Drosophila* embryos using fluorescent membrane markers. We confirm earlier reports that division angles are highly biased along the anterior-posterior axis in wild-type embryos. Interestingly, we find that division angles are significantly more random in embryos lacking anterior-posterior patterning in general and striped leucine-rich-repeat receptor expression in particular. These experiments address whether oriented cell divisions during germband extension are cell-autonomously regulated by striped gene expression or non-autonomously influenced by the distribution of mechanical tension mediated by head-to-tail patterning.

677S **Bruno1 isoforms have distinct subcellular localization patterns in developing indirect flight muscle of *Drosophila*** Sienna N Ficken[1], Erin Kelleher[2], Mainak Bose[3], Anne Ephrussi[3], Maria Spletter[1] [1]University of Missouri Kansas City, [2]University of Houston Texas, [3]The European Molecular Biology Laboratory (EMBL)

Striated muscle development in *Drosophila melanogaster* is regulated by a variety of distinct transcription and splicing regulation factors, including the RNA-binding protein Bruno1 (Bru1, also called Arrest, Aret). Bru1 is a conserved member of the CELF family of RNA binding proteins, which are key regulators of alternative splicing and muscle development in vertebrates. CELF1-2 are reported to promote embryonic splicing patterns in striated muscle and have increased activity in adult patients with myotonic dystrophy type 1 (DM1), in part due to increased nuclear localization and a reversion to embryonic splicing patterns. In flies, Bru1 instructs indirect flight muscle (IFM)-specific alternative splicing, sarcomere growth, and myosin contractility. *bru1* mutant IFM fails to undergo a developmental transition to mature splice isoforms. Interestingly, *bru1* itself undergoes alternative splicing to produce at least 11 transcripts and 6 distinct proteins. Here we examine the subcellular localization of individual Bru1 isoforms across IFM development. Using confocal microscopy, we show that during early stages of IFM development, Bru1^{eGFP} protein is localized to the cytoplasm as well as the nuclei. As the IFM matures, Bru1^{eGFP} progressively is cleared from the cytoplasm and by 72 h after puparium formation (APF) is strongly enriched in the nucleus. We use *UAS-GFP-Bru1* constructs to assay the subcellular localization of individual Bru1 isoforms including A, B, and D at early and late timepoints, and find isoform-specific patterns of subcellular localization in IFM. Intriguingly, CELF proteins in vertebrates are known to shuttle between the cytoplasm and nucleus due to PKA-mediated phosphorylation, and Bru1 in flies is also reported to be phosphorylated by PKA near the N terminus, on a serine that is not present in all isoforms of Bru1. Here we test if Bru1 nuclear localization in IFM is PKA-dependent, using RNAi knockdown and overexpression to modulate PKA activity. This research identifies regulation of Bru1 protein localization as a conserved mechanism to regulate CELF-protein activity, and suggests that different Bru1 isoforms may play distinct roles during muscle development.

678S **Identification of novel *akirin*-interacting loci** Camille Santana, Scott J Nowak Molecular and Cellular Biology, Kennesaw State University

Akirin is a nuclear cofactor involved in the gene regulation of embryonic heart patterning in *Drosophila melanogaster*. During development, Akirin facilitates gene expression by integrating Twist transcription factor activity with chromatin remodeling machinery to facilitate the proper level of Twist-regulated gene expression. Our data indicates that Akirin regulates cardiac and skeletal muscle patterning through interactions with both SWI/SNF-class and NuRD/CHD4- class chromatin remodeling complexes. Excitingly, this mechanism appears to be conserved from insects to mammals and other metazoans. We are employing a combination of forward genetic screens paired with live imaging analysis of cardiac function to uncover novel genetic loci that may work with Akirin during skeletal and cardiac patterning. To date, we have uncovered several loci that appear to fit these criteria. Our current work centers on two of these candidate loci, *hyd* and *Ppn*, both of which appear to interact with *akirin* during these developmental events. The identification of these novel Akirin interactors will yield new insights into the mechanisms by which cofactors such as Akirin are critical for developmental processes.

679S **Investigating the Function of CG1907 in Larval Tracheal Growth** Alexander J Muller, Robert E Ward Biology, Case Western Reserve University

Allometric growth is an important phenomenon in human development that explains why certain parts of our body grow to different sizes. We can investigate allometric growth using Drosophila melanogaster; during larval stages, tracheal cells stop dividing, but the trachea continues to grow and extend throughout the organism as it continues to grow. We know from previous work that tracheal-specific cell growth is under genetic control. This project characterizes a novel larval lethal mutation (known as l(3)12265) that results in an overgrown and highly convoluted trachea phenotype. Deficiency mapping indicated that the gene is located in 99B-D on the third chromosome. A compelling candidate for the gene is CG1907. CG1907 encodes a transmembrane transporter for inorganic ions. RNA interference was used to knock down CG1907 both throughout the body and specifically in the trachea through genetic crosses with Daughterless and Breathless Gal4 drivers. Ubiquitous expression of CG1907 interference caused the relative trachea length, measured by analyzing the ratio of the tracheal dorsal trunk length from the transverse connective in the fourth abdominal segment to the posterior spiracles versus total body length, to be statistically larger than the wildtype trachea. Next, antibody staining was conducted to view the relative level of Uninflatable (Uif), a gene involved in tracheal growth, in both wildtype and in the l(3)12265 allele. Quantitative confocal analysis revealed that the levels of Uif are increased in the l(3)12265 mutant flies. We are currently testing if Uif levels are also increased in da>CG1907 trachea. Quantitative RT-PCR testing will be conducted to further elucidate if increased RNA expression of uif is responsible for creating this phenotype. Lastly, a P-element excision will be conducted to generate a mutant alleles of CG1907 to determine if they fail to complement l(3)12265 and to examine how the loss of this gene impacts tracheal growth.

680S **Understanding circular invagination of epithelium using anterior midgut in *Drosophila*** Durlin U Valle, Lingkun Gu, Mo Weng University of Nevada - Las Vegas

Diverse types of cell shape change promote 3D tissue formation during embryogenesis and are mediated by force-producing machinery and cell-cell adhesion. However, except for a few well-studied models, mechanisms of many tissue shape changes remain poorly understood. We identified Drosophila ectodermal anterior midgut (ectoAMG) invagination as a novel model system to study the circular internalization of an epithelial sheet. We identified the patterning genes that define this uncharacterized tissue: ectoAMG expresses both Huckebein and Snail and is immediately anterior to the ventral stripe of Giant. Using live imaging, we show that ectoAMG cells undergo a circular invagination characterized by moderate levels of apical contractile myosin while experiencing low levels of adherens junctions. Despite a circular internalization, ectoAMG does not display myosin cables at tissue boundaries. We found that ectoAMG invagination requires concertina and T48 signaling pathways which are known to activate myosin during the apical constriction of fly ventral furrow. Despite the similar requirement of these two pathways and the physical proximity, the invagination of ectoAMG does not depend on that of ventral furrow. We will discuss the role of myosin and adherens junctions during ectoAMG invagination. We found that ectoAMG displays diverse cell shape changes and we will demonstrate the cell shape dynamics through cell tracking and 3D reconstruction. This research will offer new insights into the molecular and cellular mechanisms of similar cell shape changes in tissue formation.

681S **Non-canonical role of septate junctional proteins in border cell migration of Drosophila** Amita Nanda, Hansaem Gook Biology, Case Western Reserve University

Border cell migration (BCM) during oogenesis in Drosophila is a well-established model of collective cell migration that is applicable to understanding tissue morphogenesis, wound healing, and cancer metastasis. During mid-oogenesis, polar cells in the follicular epithelium secrete signaling molecules that recruit nearby somatic follicle cells to surround the polar cells and delaminate into the interior of the egg chamber. This cluster migrates between nurse cells to the anterior side of the oocyte. The border cell cluster demonstrates anterior-posterior, apical-basal, and rotational polarity. Our main interest is in discovering and understanding the roles of septate junction (SJ) proteins in BCM. Similar to tight junctions in vertebrate epithelia, the SJ form an occluding junction to block the paracellular flow of solutes across the membrane. Interestingly, SJs have not yet formed in the follicular epithelium at the time that BCM is occurring. Our previous work on the function of SJ proteins during embryogenesis suggest that SJ proteins might play a non-junctional regulatory role in morphogenesis. To quantify the effect of SJ protein knockdown on BCM, we employed the GAL4/UAS system, using the *slbo-Gal4* line along with RNAi to target Kune-kune (Kune), Macroglobulin complement-related (Mcr), and Coracle (Cora). Antibody staining revealed that the knockdown of Kune, Cora, and Mcr resulted in variably penetrant phenotypes including incomplete border cell migration, failure of the cell cluster to delaminate, and dissociation of the cluster during migration. Potential hypotheses are that SJ proteins may contribute to the actual polarity and orientation of border cells in order to migrate to their destination, adhesion of the border cell cluster, or movement of the cluster via actin/myosin contractions. To further investigate the functions of SJ proteins during BCM, we are performing fixed tissue and live imaging of wildtype and KuneRNAi knockdown egg chambers to observe cell shapes, polarity, adhesive structures and cytoskeletal dynamics. The identification of adhesion or polarity markers which differ between SJ mutant and control BC clusters may provide a pathway for future research to better understand mechanisms of collective cell migration in a variety of tissues and organisms.

682S **Palmitoyltransferases and Golga7 in Fat/Dachsous mediated growth control in Drosophila** Alex Murphy[1], Xing Wang[2], Seth Blair[1] [1]UW-Madiison, [2]Beijing Key Laboratory of Biodiversity and Organic Farming

Heterophilic protocadherins Fat and Dachsous (Ds) and their downstream effectors regulate growth and planar cell polarity by modulating the levels and localization of Dlish, a SH3-containing protein, and its binding partner, the atypical myosin Dachs. Loss of Fat or its intracellular domain (ICD) increases Dlish/Dachs levels at the subapical cortex where Dachs negatively regulates Warts, the final kinase of the Hippo pathway. Conversely, subapical accumulation of Dachs/Dlish requires the DHHC palmitoyltransferase (DHHC) Approximated (App). App palmitoylates Dlish and the Fat ICD, tethering Dachs/Dlish to the subapical cortex and weakening Fat's impact on Dachs/Dlish localization. Fat ICD palmitoylation persists in *app* null mutants, suggesting the involvement of additional DHHCs. Most of Drosophila's 20 DHHCs concentrate in the Golgi or ER, but dZDHHC8, like App, can localize to the plasma membrane. We assessed dZDHHC8's effects on spacing between the crossveins (CV) in adult wings, as reductions are common in mutants of Fat/Ds pathway components, including App knockdowns. We found that dZHHC8 knockdown weakly reduced CV spacing in WT and *app* null mutants, indicating its involvement in the Fat/Ds pathway, potentially acting redundantly to App. We also found that dZDHHC8 (and App) binds Dlish though little is known about their regulation. App and dZDHHC8 resemble yeast Erf2 and mammalian ZDHHC9 and 5, which bind GOLGA7/Erf4 cofactors thought to aid in target recognition and palmitoylation. We found that the sole Drosophila GOLGA7 (CG5447) is required for normal CV spacing and WT accumulation of Dachs/Dlish. Knockdown of dGolga7 also strongly reduced App and dZDHHC8 levels. This suggests dGolga7 regulates the Fat/Ds pathway through control of DHHC stability. We observed GOLGA7-DHHC binding *in vitro* and are testing conserved binding motifs to provide tools for further analyses. Supported by NIH R01GM151072 and the UW-Madison Genetics Training Program

683S ***In vitro* Reconstitution of FGF Signaling Demonstrates Differential Activation of Heartless by Homologous FGFs Pyramus and Thisbe** Vincent Stepanik[1], Angelike Stathopoulos[2] [1]Biology and Biological Engineering, California Institute of Technology, [2]California Institute of Technology

Fibroblast Growth Factor signaling functions through the binding of secreted ligands (FGFs) to membrane-bound receptors (FGFRs). In *Drosophila melanogaster*, the duo of FGF ligands Pyramus (Pyr) and Thisbe (Ths) are dedicated to one FGFR, Heartless (Htl). This complement of proteins is required for cell behaviors conserved among FGF signaling outputs, such as migration of mesodermal derivatives, specification of pericardial tissue, and control of apoptosis. The expression patterns of Pyr and Ths have overlapping yet unique spatiotemporal profiles, and both have unique protein domains outside of their receptor-binding domains. Together, this suggests that each harbors intrinsic elements facilitating specific cellular outputs through Htl.

To test Pyr and Ths for such differences, we developed an *in vitro* model of FGF signaling using S2 cells. S2 cells do not natively express Htl, Pyr, Ths, or the Htl signaling adapter protein Downstream of FGF (Dof)/Stumps that is required for MAPK activation via Htl *in vivo*. Delivery of soluble Pyr or Ths to S2-Htl+ cells results in MAPK activation. Co-incubating Pyr+ or Ths+ cells with S2-Htl+ cells to better recapitulate how the ligands are presented *in vivo* yields similar results. Pyr causes a higher initial peak of MAPK activation, while Ths induces a more sustained MAPK output. Each ligand also induces unique morphological changes in S2-Htl+ cells.

Curiously, we find that the adapter Dof is not required for MAPK phosphorylation via Htl despite its requirement *in vivo*. Rather, Dof alters the dynamic range of MAPK activation, lowering baseline MAPK phosphorylation while increasing its maximal level. This suggests the dependence of Htl-dependent MAPK phosphorylation on Dof *in vivo* is not due to its presence per se, but rather a function of signaling kinetics afforded by Dof, or that Dof antagonizes negative regulation of Htl function *in vivo* that is absent from S2 cells.

Our *in vitro* model of FGF signaling shows that activation of Htl by Pyr is not equivalent to that by Ths, despite activating the same downstream signal, and that the role of Dof is more likely regulatory than obligatory. Future experiments will address the underlying mechanisms.

684S **Spatial and temporal expression analysis of patterning and structural pigmentation genes using hybridization chain reaction (HCR) in *Drosophila* pupae** Erick X Bayala Rodriguez, Patricia J Wittkopp MCDB, University of Michigan

Research in flies has been crucial in helping us understand the genetic and developmental basis of pigmentation. Such research has provided information on what genes are involved with both the patterning and pigment synthesis stages of pigmentation. Details on the expression of many of these patterning genes and pigmentation effectors are mostly restricted to specific time points and obtained by different techniques that are only sometimes comparable, compatible, or to a degree, scalable. Because of this, many gaps exist in our current understanding of the temporal and spatial dynamics of the expression windows of genes involved with pigmentation. Such gaps make it difficult to understand pigmentation (including its patterning) as a continuous process during development or to answer more mechanistic questions. To fill such knowledge gaps, new techniques must be developed or applied to investigate simultaneously the expression of multiple genes involved with pigmentation across the entire development of the fly (across multiple tissues and within different species). Here we report details on our newly designed protocols for the dissection and staining (via HCR) of epidermal tissue across the entire fly and within different fly species. In *D. melanogaster*, we have simultaneously observed the expression of the patterning gene *omb* in addition to the pigmentation effectors, *yellow*, *ebony*, and *tan* helping us understand and map their expression dynamics during most of pupal development. We also have applied the same protocols to other non-*melanogaster* species allowing us to map and analyze expression differences (spatially and temporally) for the same genes.

685S **Differential expression of the homophilic cell adhesion molecule Echinoid leads to localized actomyosin contractility that drives epithelial morphogenesis.** Matthis Blanchard, Arsida Noçka, Rahul P Rote, Laura Nilson Biology, McGill University

During development, tissue morphogenesis relies on various cellular behaviors, such as cell movements and shape changes, that are regulated in space and time. Cells must sense and interpret signals from their environment and coordinate with neighboring cells. We have shown previously that the dynamic expression of the protein Echinoid (Ed), a homophilic cell adhesion molecule and putative adherens junction component, provides a spatiotemporal cue that drives morphogenesis in two distinct developmental contexts: dorsal closure in late embryogenesis and epithelial tube formation in the follicular epithelium. In each case, the developmentally programmed loss of Ed from a defined population of cells generates an interface between cells without Ed expression and adjacent cells that maintain Ed expression. These “Ed/no-Ed” interfaces have a smooth contour, are enriched for markers of actomyosin contractility, and are required for morphogenesis. These interfaces also lack Ed entirely because the no-Ed cells do not provide a homophilic binding partner, leading to the planar polarization of Ed on the Ed-expressing side of the interfaces. In these polarized cells, a contractile actomyosin cable forms where Ed is absent, driving cell shape changes and coordinating tissue morphogenesis. These findings uncover a role for differential expression of Ed in generating localized contractility that drives morphogenesis, but how Ed transfers this information to the cytoskeleton remains unknown.

To address this question, we focused on the Ed intracellular domain. When cells expressing a transgenic form of Ed that lacks the intracellular domain (Ed-ΔC) are juxtaposed with no-Ed cells, Ed-ΔC is still planar polarized but actomyosin enrichment and the smooth contour are abolished. The Ed intracellular domain is thus essential for Ed function in this context, but sequence analysis did not reveal any obvious functional domains. We therefore tested a series of Ed transgenes, bearing deletions in the Ed intracellular domain, for their ability to produce a smooth contour. This analysis revealed a region of Ed that is essential for this phenotype and might interact with actin regulators.

This work suggests that the homophilic binding properties of the Ed extracellular domain allow it to sense the presence or absence of Ed in adjacent cells, while the Ed intracellular domain translates this spatial information into localized actomyosin contractility that drives morphogenesis.

686S **An optogenetic approach to define cytoskeletal contributions to assembly of the testis stem cell niche** Everette Rhymer, Lauren Anllo Biology, East Carolina University

To retain their undifferentiated state, stem cells rely on self-renewal signals from their cellular microenvironment, the niche. A functional niche is often assembled in a specific tissue region to limit renewal signals to a select number of stem cells. Studying how niche assembly is coordinated with organogenesis is imperative to understanding regulation of tissue homeostasis and regeneration. We leverage the *Drosophila* testis to investigate development of its niche. Prior to niche assembly, the embryonic male gonad is a spherical arrangement of germ cells interspersed with somatic cells, a subset of which are specified as pro-niche cells. During assembly, pro-niche cells migrate to the outer edge of the gonad, and then to the anterior, where they cluster into a smooth, circular niche (Anllo et al., 2019). An unassembled niche cannot signal properly to GSCs. It is therefore crucial to understand mechanisms that underly niche formation. We previously showed that niche assembly requires niche cell accumulation of the transcription factor Islet, which is required for niche cells to polarize their cytoskeleton (Anllo & DiNardo, 2022). What remains elusive is whether F-actin directly mediates assembly, and what specific regulators of F-actin are required for the assembly process. We have established a method that employs molecular optogenetics to manipulate F-actin regulatory mechanisms during specific stages of niche development. Here, we demonstrate precise temporal control of the Cry2 CIBN system in the embryonic testis, using blue light to direct localization of cytoskeletal modulators to the cell cortex. Using *in vivo* live imaging with these optogenetic techniques, we show a direct role for cortical F-actin in niche assembly. We also use our method to show a requirement for the cytoskeletal contractility modulator Rho1 in both the assembly and cytoskeletal polarization of niche cells. We further developed a method to employ fixed-tissue immunostaining post live photoactivation, enabling us to show that Rho1 activity is required for niche cells to accumulate the adhesion protein and niche cell identity marker FasciclinIII. Our data suggest a role for cytoskeletal contractility in modulating cell adhesions during niche assembly. Our work represents a novel method to define cytoskeletal contributions required for niche assembly that will elucidate regulatory mechanisms broadly applicable to niche formation.

687T **The ubiquitin-conjugating enzyme UBE2D/eff maintains a youthful proteome and ensures protein quality control during aging by sustaining proteasome activity** Fabio Demontis DNB, St. Jude Children›s Research Hospital

Ubiquitin-conjugating enzymes (E2s) are key for protein turnover and quality control via ubiquitination. Some E2s also physically interact with the proteasome, but it remains undetermined which E2s maintain proteostasis during aging. Here, we find that E2s have diverse roles in handling a model aggregation-prone protein (huntingtin-polyQ) in the *Drosophila* retina: while some E2s mediate aggregate assembly, UBE2D/effete (eff) and other E2s are required for huntingtin-polyQ degradation. UBE2D/eff is key for proteostasis also in skeletal muscle: eff protein levels decline with aging, and muscle-specific eff knockdown causes an accelerated buildup in insoluble poly-ubiquitinated proteins (which progressively accumulate with aging) and shortens lifespan. Mechanistically, UBE2D/eff is necessary to maintain optimal proteasome function: UBE2D/eff knockdown reduces the proteolytic activity of the proteasome, and this is rescued by transgenic expression of human UBE2D2, an eff homolog. Likewise, human UBE2D2 partially rescues the lifespan and proteostasis deficits caused by muscle-specific eff^{RNAi} and re-establishes the physiological levels of eff^{RNAi}-regulated proteins. Interestingly, UBE2D/eff knockdown in young age reproduces part of the proteomic changes that normally occur in old muscles, suggesting that the decrease in UBE2D/eff protein levels that occurs with aging contributes to reshaping the composition of the muscle proteome. However, some of the proteins that are concertedly upregulated by aging and eff^{RNAi} are proteostasis regulators (e.g., chaperones and Pomp) that are transcriptionally induced presumably as part of an adaptive stress response to the loss of proteostasis. Altogether, these findings indicate that UBE2D/eff is a key E2 ubiquitin-conjugating enzyme that ensures protein quality control and helps maintain a youthful proteome composition during aging.

688T **The *Drosophila melanogaster* enzyme Glycerol-3-phosphate dehydrogenase (GPDH1) interacts with Target of rapamycin (Tor) to regulate brain growth.** Shefali A Shefali, Madhulika Rai, Jason Tennessen Biology, Indiana University Bloomington

Animal development requires integration of nutritional cues with signaling pathways and gene expression networks. Thus, nutrient-sensing proteins play an essential role in coordinating nutrient availability with growth and maturation. Although substantial work has been conducted on the endocrine regulation of carbohydrate metabolism, questions remain about how cells sense and coordinate changes in glucose flux across different organs and cell types. Here we investigate this question using the fruit fly, *Drosophila melanogaster*, which is ideally suited for studying interorgan communication. My work reveals a novel mechanism by which glycerol-3-phosphate dehydrogenase 1 (GPDH1), a key enzyme that regulates abundance of the glycolytic intermediate dihydroxyacetone phosphate (DHAP), interacts with the nutrient sensor Target of rapamycin (Tor) to control brain growth. We have found that Gpdh1 – Tor signaling crosstalk is prominent both in the adipose tissue and within the neural stem cells, and when dysregulated causes metabolic imbalance - leading to impairment in brain mitotic activity and ectopic brain growth. Due to chronic alterations in the cell cycle of neural stem cells caused by disruption of Gpdh1-Tor signaling system, the brain experiences excess cell proliferation and ultimately becomes tumorous. As an extension of these studies, we have also discovered that exposure to metabolic stresses including nutrient restriction and hypoxia, leads to a systemic increase in GPDH1 expression. Our data suggests that Tor - GPDH1 signaling crosstalk is a possible metabolic stress response mechanism where Tor inhibition causes an increase in GPDH1 expression, which depletes the DHAP pool and further suppresses Tor-dependent growth signaling, allowing animals to optimize survival in stress conditions. Moreover, in absence of Gpdh1, accumulation of DHAP non-canonically activates Tor signaling leading to a growth resistant phenotype. Overall, our studies demonstrate how changes in glycolytic flux affects growth signaling and hold the potential to uncover a fundamental mechanism by which GPDH1 and Tor non-autonomously regulate tissue growth in response to changes in carbohydrate metabolism.

689T **Strong GAL4 expression impairs adult fat body function** Scott A Keith, Ananda A Kalukin, Dana S Vargas Solivan, Brian P Lazzaro Cornell University

The ability to direct tissue-specific expression and overexpression of transgenic proteins in *Drosophila* has facilitated innumerable biological discoveries. However, transgenic proteins can themselves impact fly physiology, in ways that are often ignored or poorly defined. In adult flies, the fat body regulates major physiological functions, including energy metabolism, innate immunity, and, in females, provisioning developing oocytes. The yolk-GAL4 transgene is commonly used to conduct genetic manipulations in the fat body, as the yolk enhancer sequence directs GAL4 expression specifically in females and only during adulthood. We found that flies heterozygous for yolk-GAL4 exhibited significant physiological defects. Flies carrying yolk-GAL4 produced extremely low numbers of eggs and were highly susceptible to systemic bacterial infections. These phenotypes suggested impaired fat body function, and we found that fat bodies of yolk-GAL4-expressing flies contained morphologically degenerated adipocytes and reduced lipid levels. Importantly, all of these defects were fully suppressed when we knocked down GAL4 production in yolk-GAL4 heterozygotes using RNAi, thus confirming these phenotypes result from expression of the transgene. We hypothesized that GAL4 dosage might determine the severity of functional impairment. Consistent with this, we observed a striking, direct correlation between GAL4 expression levels and susceptibility to systemic infection across multiple commonly used fat body driver lines. We then asked whether these physiological impairments are a general consequence of either high-level expression of foreign proteins or overwhelmed nuclear import, or if they reflect toxic effects specifically caused by yeast GAL4. To test this we constructed fly lines with the yolk regulatory sequence driving expression of either cytoplasmic or nuclear-localized mCherry. We found that nuclear-localized but not cytoplasmic mCherry caused infection susceptibility comparable to yolk-GAL4, but did not impair egg laying or fat storage. As an independent test, we also used homology-assisted CRISPR knock-in to convert yolk-GAL4 to yolk-lexA, and found that expression of lexA, another nuclear-localized transcription factor, also disrupted infection resistance but not fecundity or fat tissue integrity. These results suggest that basal fat body functions including energy storage and egg provisioning may be sensitive to high levels of GAL4 protein specifically, while the capacity to mount rapid, intense responses to environmental challenges like infection can be more generally disrupted by overwhelmed nuclear-import.

690T **Con-FLIC and Con-DAM: Platforms for the Concurrent Measurement of Feeding Behaviors, Sleep, and Food Intake at Single-Fly Resolution in *Drosophila melanogaster*** Mubaraq Opoola, Breanna Beard, Dae-Sung Hwangbo Biology, University of Louisville

Accurate quantification of food intake and feeding behavior is essential for understanding various physiological and behavioral processes, such as metabolism, sleep, and aging. While fruit flies have been widely used to study these processes across diverse disciplines—from ecology and evolutionary biology to molecular genetics—precisely measuring food consumption, feeding-related behaviors, and sleep in the same individual fly has been challenging. We have developed new tools, termed Con-FLIC and Con-DAM, which integrate the Consumption-Excretion (Con-Ex) method with the Drosophila Activity Monitor (DAM) system and the Fly Liquid-food Interaction Counter (FLIC), respectively, into a single platform. At single-fly resolution, the Con-DAM enables the measurement of food consumption and various sleep parameters, while the Con-FLIC allows for the quantification of food consumption with various feeding-related parameters both at the single fly resolution. These tools offer a straightforward solution for simultaneously quantifying food consumption and sleep/feeding patterns in individual flies and small insect models over an extended period.

691T **Identifying Novel Regulators of Food Consumption through Genome-Wide Association Study and Chemoconnectome Screening in *Drosophila*** Mubaraq Opoola[1], Makayla Wright[2], Lucas Fitzgerald[1], Dawson Sheble[3], Abigail Rutledge[1], Dae-Sung Hwangbo[1] [1]Department of Biology, University of Louisville, [2]University of Kentucky, [3]Biology, Eastern Kentucky University

Food consumption is essential to physiological and behavioral processes like aging, health, reproduction, and many more. These processes are affected by the quality and quantity of food ingested. Overconsumption of food, especially sugar, can lead to obesity, a precursor to diseases such as type 2 diabetes, heart disease, and reproductive deficiencies. Understanding the genetic and neuronal mechanism by which food consumption is regulated is essential to prevent these diseases. To elucidate these mechanisms, we performed Genome-wide Association Studies (GWAS) of ~200 Drosophila Genetic Reference Panel (DGRP) lines– a set of fully sequenced inbred lines derived from a natural population -, and screening of ~150 genes in the chemoconnectome (CCT)– the entire set of neurotransmitters, neuromodulators, neuropeptides, and their receptors underlying chemotransmission in an animal. We identified many key genes regulating food consumption in Drosophila. Among these genes are many G protein-coupled receptors like methuselah-like genes (mthl), metabotropic GABA-B receptor subtype 2 (GABA-B-R2), adenosine receptor (AdoR), adipokinetic hormone receptor (AkhR) and neuropeptides like prothoracicotropic hormone (ptth), CCHamide-1 (CCHa1) and adipokinetic hormone (Akh). Our findings provide more insight into the highly polygenic genetic architecture of food consumption.

692T **Roles of the fat body circadian clock in diet restriction mediated changes in sleep and starvation response** Breanna Beard[1], Aubrey Reitzel[2], Bailey Ramirez[2], Krish Patel[2], Dae-Sung Hwangbo[2] [1]Biology, University of Louisville, [2]Department of Biology, University of Louisville

Diet restriction (DR), or the reduction of nutrients not to the point of malnutrition, is one of the most robust non-genetic interventions for increasing lifespan and healthspan in a variety of model organisms. While the molecular mechanisms through which DR delays aging are unknown, emerging evidence suggests a strong role for the circadian clock in regulating longevity. Additionally, clocks are implicated in regulating DR-mediated changes in behavior and physiology, but how this contributes to aging is unknown. Circadian clocks are endogenous free-running oscillators that produce 24-hour rhythms to maintain behavioral and physiological homeostasis through temporal changes in clock-controlled genes (CCGs). The Drosophila fat body is analogous to the mammalian liver and adipose tissue and has its own autonomous clock system. Our RNA-sequencing data revealed diet-dependent reprogramming of the circadian transcriptome in the peripheral fat body. Therefore, we hypothesize that some of these CCGs mediate diet-dependent changes in sleep and starvation resistance. To identify the CCGs in the fat body that are required for DR-mediated changes in sleep and starvation response, we employed a tissue-specific RNAi screen by suppressing over 100 top cycling genes in the fat body. Our results indicate that fat body CCGs regulate starvation resistance in diet-dependent and diet-independent mechanisms, while sleep is largely not influenced by the fat body clock. Overall, these findings provide critical insights into how clocks in the periphery regulate behavior and physiology.

694T **Regulation of metabolic sexual dimorphisms in larvae** Arely V Diaz, Tânia Reis Department of Medicine, University of Colorado Anschutz Medical Campus

My work has measured metabolic differences in larvae—which are sexually immature—and explored roles for Spenito (Nito) and the sex determination pathway in these differences. Previously, we discovered that Nito is required for proper fat storage, and that there are sex differences in fat levels at the larval stage with males having higher fat levels than females. Nito is also required for proper sex determination via regulation of alternative splicing in the canonical *Sex-lethal* pathway. To investigate the role of Nito in metabolic dimorphism I measured fat differences in larvae with FB-specific Nito knockdown. I found abolished fat differences between males and females in larvae lacking Nito in their fat cells and differential expression of several metabolic genes. We further showed that Nito is required for regulating the sex-dimorphic expression of these genes and that misregulation correlates with the lack of male versus female fat differences. We predict that Nito-dependent m^6A modification of transcripts encoding key metabolic enzymes results in dimorphic expression and ultimately metabolic differences. We knocked down other members of the m^6A complex. FB-specific knockdown of three other members of the complex also made larvae lean, but the differences between the sexes were mostly preserved. The lean phenotype resulting from Nito depletion in the FB, and the observed differences in fat metabolism between male and females could reflect differential m^6A modification of metabolic targets. To directly test this prediction, we are analyzing transcriptome-wide m^6A differences in the FB of males versus females. Specifically, I am implementing a technique called eTAM for the absolute quantification of the m^6A methylome. This work will determine the mechanistic details of how Nito, through its m^6A activity, differently processes target RNAs in the FB of male versus females to establish fat dimorphism. In parallel to the role of Nito and m^6A modification, we are also investigating the role of the sex determination pathway in regulating fat dimorphism. We have previously shown that downstream target of Sxl, *transformer* (*tra*), also regulates dimorphism in metabolic gene expression intrinsic to the larval FB. Currently, my work is focused on uncoupling sex determination and Nito-dependent regulation of fat dimorphism. Given that larvae are sexually immature, we are also investigating the biological implications on fertility of these pathways. Altogether this will elucidate the mechanism by which Nito regulates metabolic dimorphism in larvae.

695T **Immediate and long-term gene expression changes in response to exercise** Eric Randolph[1], Nicole C Riddle[2] [1]University of Alabama at Birmingham, [2]Biology, University of Alabama at Birmingham

Exercise is recommended to patients suffering from a range of diseases, including cardiovascular disease, diabetes, and even cancer. Despite the fact that public health officials promote exercise for patients across the world as part of a healthy lifestyle, how exercise precipitates its many health benefits is not clear. To investigate both short-term and long-term effects of exercise, we used *Drosophila melanogaster*. We utilized the Treadwheel to induce exercise in the animals by rotating their enclosures. Comparing the response of four wild-derived strains from the Drosophila Genetics Reference Panel to early life exercise with sedentary controls, we find that the physiological responses strongly depend on sex and genotype of the animals. In our study, treatment effects that act across all groups are absent. We detected physiological effects immediately following the exercise treatment, but also effects that are either maintained for long-term or only detected in older animals. Investigating the gene expression responses to the exercise treatment, we identified several hundred differentially expressed genes associated with exercise. Interestingly, the transcriptome responses were highly sex-specific, with females and males sharing few exercise-associated differentially expressed genes. Exercise reduces the number of age-associated differentially expressed genes, suggesting that despite the limited physiological response, exercise might promote a more youthful expression profile. On-going studies are investigating how the length of the exercise treatment impacts responses.

696T **A specialist species of the floridosa group, *Drosophila lutzii*, shown distinctive metabolism and behavior from generalist species of *Drosophila*** Juan M. 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 made a comparative study between *Drosophila lutzii,* a specialist, and sympatric *Drosophila simulans*, a generalist. *D. simulans* is a saprophytic generalist, with feeding based on rotting plants and fruits, while *D. lutzii* is a phytophagous specialist. We have found *D. lutzii* eggs, larvae, pupae and adults inside *Ipomoea sp.* flowers. This suggested a restricted diet, and thus, an interesting avenue for research in metabolism, in comparison to generalist species of flies. We found that freshly caught *D. lutzii* from the wild have higher total carbohydrates and glucose levels, but similar total lipid and triglycerides 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 species also showed a differential feeding behavior when exposed to food with different amounts of sugar. Taken together, our results show that, in contrast to generalists, this specialist species, with more restricted habitat and feeding, is less capable of metabolic and feeding behavior adjustments.

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697T **The Impact of Intestinal Occluding Junction Modulation on Aging and Disease** Latrell Fomby, Samantha Le, David Grace, Emma Sebastian, Anna Salazar Christopher Newport University

Aging is a process marked by a continuous decline in multiple physiological functions, including the intestinal barrier function, which is tightly linked to longevity in *Drosophila melanogaster* and other organisms. Previous experiments have revealed 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, improves intestinal barrier function during aging, limits dysbiosis, and extends lifespan. 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. This project will investigate the impact of the gut on tissue outside of the gut and address communication between the gut and the brain and muscles in disease models. Current work utilizes cellular and molecular biological methodologies to build upon current knowledge to address crucial questions at the intersection between microbial dysbiosis, epithelial integrity, inflammation, protein aggregation, neurodegeneration, and disease, with the ultimate goal of discovering novel therapies that may enhance barrier function, healthspan, and lifespan

698T ***Lamp1* deficiency differentially affects lipid regulation in larval fat bodies and midgut and causes lipid transport defects** Sumit Gautam[1], Norin Chaudhry[2], Emily Lindgreen[3], Prasoon Jaya[4], Anna Schwake[2], Andreas Jenny[4], Gustavo Macintosh[2] [1]Biochemistry and biophysics, iowa state university, [2]iowa state university, [3]Iowa state university, [4]Albert Einstein College of Medicine

Lysosomes participate in macromolecule turnover, storage and degradation of metabolites, as hubs that integrate nutritional signals, and regulators of lipid metabolism. We characterized Drosophila Lamp1, an abundant lysosome membrane protein. Among other phenotypes, *Lamp1* mutants have increased levels of diacylglycerols with mid-chain fatty acids, suggesting defects in interorgan lipid transport. We hypothesized that defects in lipid transport should cause additional, tissue-specific cellular and molecular phenotypes. Microscopy analyses of WT and *Lamp1* larvae showed increased accumulation of neutral lipids in the anterior midgut of the mutant, with increase in lipid droplet (LD) size. In contrast, no difference in LD size was observed in fat bodies (FB). In addition, *Lamp1* enterocytes have shorter microvilli. We then examined changes in the FB and midgut proteomes caused by Lamp1 deficiency. Many proteins associated with lipid metabolism were differentially affected. Concurrent with the increase in LD size in midgut, Jabba and RDH1, two LD-associated proteins, were significantly elevated in this tissue, and a significant decrease in Jabba accumulation was observed in Lamp1 FBs. Additionally, Lamp1 midgut proteome indicated increases in TAG remobilization and FA oxidation, and decrease in FA synthesis, while a decrease in TAG/FA oxidation was observed in Lamp1 FB. Strikingly, a large increase in SNF4Aγ in Lamp1 midgut and decrease of the protein in Lamp1 FB was observed. Our results indicate that Lamp1 is necessary for lipid metabolism, facilitating export of lipids from the midgut to other tissues. Its deficiency leads to tissue-specific AMPK-dependent compensatory changes, likely needed to maintain lipid homeostasis.

699T **Growth by breakdown: glial sphingolipid catabolism fuels brain maturation** John Vaughen[1], Emma K Theisen[2], Irma Magaly Rivas-Serna[3], Taylor Jay[4], Vera Mazurak[3], Thomas Clandinin[3], Tom Clandinin[2] [1]Anatomy, University of California San Francisco, [2]Stanford, [3]University of Alberta, [4]Vollum Institute

Lipids support cell-type and organelle functions across multiple timescales, ranging from acute synaptic vesicle dynamics to slower membrane turnover during sleep. Brains harbor thousands of lipid species, yet how the lipidome is assembled, maintained, and functions remains unclear. One major group of lipids are sphingolipids, which underlie numerous neurodegenerative diseases. Here, we use *Drosophila* to manipulate sphingolipid enzymes and find that electrically active brains coordinate neural-glial metabolic networks to produce sphingolipids critical for glial morphology and synapse maturation.

To test how lipids underpin neurodevelopment, we profiled major sphingolipids and phospholipids during brain development. Contrary to our expectations, the strongest period of lipid flux occurred after neural wiring and concomitant with electric activation. Beginning at 50% pupal development and ramping from 75% to eclosion, profound remodeling of lipid tails occurred, with sphingolipids elongating and phospholipids desaturating. To block sphingolipid biosynthesis, we removed the rate-limiting enzyme *lace* in all neurons, all glia, or both cell types. Remarkably, while *lace*^{KD} in neurons or glia did not trigger obvious deficits, dual depletion caused sphingolipid depletion, protein aggregates, and lethality post-eclosion. Lipidomics on *lace*^{KD} brains pinpointed distinct alterations and compensatory signatures that imply that glia and neurons synthesize discrete pools of sphingolipids.

In one model of lipid compensation, biosynthetic enzymes are secreted. In another, lipids are nonautonomously repurposed via lysosomal salvage. Combining mutants that block sphingolipid catabolism in lysosomes (*Gba1b* or *Sap-R*) with *lace*^{KD} in glia (but not neurons) phenocopied neural+glial *lace*^{KD} protein aggregates. Thus, neural lipids can rescue glial lipid biosynthetic defects via catabolic salvage pathways. Implicating neural membrane phagocytosis, we detected Draper within aggregates in *lace*^{KD} brains. Moreover, co-depleting the phagocytic effector kinase *Shark* with *lace* in glia triggered similar aggregates. We traced protein aggregates to a subclass of neuropil-associated glia expressing glutamine synthetase (Gs2+). Blocking downstream sphingolipid biosynthetic enzymes for Cer or CPE (*schlank* or *cpes*) caused cell-autonomous aggregates in Gs2+ glia and improper infiltration during later development. Moreover, *cpes* removal in glia significantly reduced *brp::GFP* by 25%, and *cpes* removal in both neurons and glia halved *brp::GFP*. Thus, glial CPE, which can be derived from glia *de novo* biosynthesis and neural membrane catabolism, fuels brain function by supporting neuropil glial morphology and synapse assembly.

700T **The Drosophila Hypoxia-inducible factor 1-alpha is required to establish the larval glycolytic program** Tess Fasteen[1], Yasaman Heidarian[1], Liam Mungcal[1], Nader Mahmoudzadeh[1], Julie R Haines[2], Robert Pepin[1], Angelo D'Alessandro[2], Jason Tennessen[1] [1]Indiana University, [2]University of Colorado School of Medicine

The rapid growth that occurs during *Drosophila* larval development requires a dramatic rewiring of central carbon metabolism to support biosynthesis. Larvae achieve this metabolic state, in part, by coordinately up-regulating the expression of genes encoding enzymes in carbohydrate metabolism. The resulting metabolic program exhibits hallmark characteristics of aerobic glycolysis and establishes a physiological state that supports growth. To date, the only factor known to activate the larval glycolytic program is the *Drosophila* Estrogen-Related Receptor (dERR). However, dERR is dynamically regulated during the onset of this metabolic switch, indicating that other factors must be involved. Here we discover that Sima, the *Drosophila* ortholog of Hif1α, is also essential for establishing the larval glycolytic program. Using a multi-omics approach, we demonstrate that *Sima* mutants fail to properly activate aerobic glycolysis and die during larval development with metabolic defects that mimic those observed in *dERR* mutants. Further, we observed that sima and dERR protein each fail to be stabilized in the absence of the other, suggesting cooperative regulation of downstream processes. Altogether, our studies indicate that Sima, like dERR, is an essential regulator of the larval metabolic program and establishes the fly as a powerful genetic model for studying the interaction between these two ancient metabolic regulators.

701T **_Drosophila_ as a model for Precision Toxicology** Shannon Smoot[1], Jessica Holsopple[1], Alex Fitt[1], Matt Lowe[1], Thom Kaufman[1], Brian Oliver[2], Jason Tennessen[2] [1]Biology, Indiana University, [2]Indiana University

Global industries are rapidly producing and releasing tens of thousands of chemicals, yet the effects of these molecules on environmental and human health are inadequately understood. This lack of knowledge, coupled with current mammalian testing methods that are both expensive and time-consuming, leaves a dangerous knowledge gap that must be addressed using inexpensive and high-throughput models. The fruit fly *Drosophila melanogaster* has emerged as an ideal system for studying the mechanisms by which individual chemicals alter animal behavior, physiology, metabolism, and gene expression. In this regard, *Drosophila* studies are uniquely situated to quickly identify the molecular targets of individual toxicants via the use high-throughput multi-omics and an unparalleled genetic toolkit. We have screened over 200 chemical compounds for lethality and behavioral phenotypes to determine appropriate exposure conditions for metabolomic and transcriptomic analysis. Here we demonstrate the power of the multi-omic approach by analyzing adult male and female flies fed acute doses of five diverse compounds (sodium arsenite, cadmium chloride, DMSO, ethprophos, and pirinixic acid). By using a combination of transcriptomic and metabolomic methods, we identified a series of dose and time-dependent effects on metabolic pathways and gene expression networks. Our analysis revealed that chemical exposure not only activates metabolic and stress-related pathways that are known to protect flies against global toxic effects, but we also uncovered previously undescribed sex-specific responses. Notably, we observed significant changes in the expression of genes involved in oogenesis and seminal fluid protein expression, indicating that these compounds significantly affect gamete formation and function. Overall, our findings demonstrate how *Drosophila* can serve as a powerful model to identify the sex-specific genetic and metabolic response to toxicant exposure when applying a multi-omics approach.

702T **Methuselah antagonists targeted to Insulin-producing cells extend health span, in _Drosophila melanogaster_** Ravi Ranjan Health Sciences, California Nothstate University Health Sciences

Methuselah antagonists targeted to Insulin-producing cells extend health span, in *Drosophila melanogaster*

Ravi Ranjan, Lore, Al, Yang K, Gimenez LE, Hu H, Ja WW *California Northstate University Health Sciences, CA*

The novel role of G-protein coupled receptors (GPCRs) and their ligands in aging have been demonstrated by studies on Methuselah (Mth), where downregulation of *mth* or its ligands Stunted, by genetic or pharmacological manipulations, increases lifespan in *Drosophila*. How *mth* ligands function to control aging is largely unknown. An essential step towards the understanding of Mth ligands is to determine the molecular mechanisms and tissues that may require its function to extend lifespan. Here, we report that specific targeting of Insulin-producing cells (IPCs) by the antagonist in the brain extends lifespan, increases stress resistance, and improves locomotion and memory. Long-lived antagonist-targeted flies show reduced levels of insulin-like peptides (dilp2) and increased lipids and carbohydrate storage but normal fecundity. Together these analyses suggest that antagonists function in the IPCs to regulate the stress pathway components JNK and Foxo, their target genes Sod and Sirt4 and thus play a pivotal role in the determination of lifespan. I have further demonstrated that stunted ligands can also ameliorate early as well as a late human model of Alzheimer's progression.

703T **Investigating the molecular mechanisms driving lipid metabolic changes induced by intermittent, time-restricted feeding (iTRF)** Jared A Gatto[1], Timothy Chang[2], Wendy Kanmogne[2], Andres Martinez-Muniz[2], Adriana Velez-Alicea[2], Julie C Canman[2], Mimi Shirasu-Hiza[2] [1]Genetics and Development, Columbia University Medical Center, [2]Columbia University Medical Center

Today we live in an age of unprecedented access to food. Recent research suggests that many Americans eat from the time they wake up to the time they go to sleep. Unfortunately, eating at night is linked to several aging-related comorbidities, including obesity, cardiovascular disease, and type-2 diabetes. Conversely, dietary interventions that restrict eating to the day (active phase) protect many aspects of health, even without reducing caloric intake. Time-restricted feeding (TRF) diets reduce oxidative stress and inflammation, decrease insulin resistance, and lower blood sugar in both mice and humans, as well as reduce fat levels, protect against a high-fat diet, and prevent obesity in mice. Yet, the molecular mechanisms underlying the metabolic benefits of time-restricted feeding remain unclear. Using *Drosophila*, our lab developed a TRF diet that robustly extends lifespan and delays molecular signs of aging. In addition, we found that TRF reprogrammed lipid metabolism: after 10 days of TRF, TRF flies were very sensitive to starvation relative to ad lib flies and had significantly less fat relative to *ad lib* flies, as confirmed through thin-layer chromatography. TRF flies did not eat less than *ad lib* flies; in fact, TRF flies ate more overall. In addition, both males and females exhibited lipid loss during iTRF, and this fat loss was not due to the same mechanisms required for lifespan extension (autophagy and the circadian clock). To understand how TRF flies lose fat stores, we conducted an RNA-sequencing experiment over two circadian cycles, encompassing both fasting and refeeding periods at 10-days and 30-days of TRF. While analysis remains ongoing, we identified multiple metabolic genes with unexpected TRF-associated expression patterns, including beta-oxidation and lipase genes, that might explain how TRF shifts lipid metabolism away from anabolism and toward catabolism. We are currently testing these genes genetically for their role in TRF-mediated changes in lipid metabolism. We are also currently testing iTRF's therapeutic potential in alleviating high-fat levels in flies fed a high-sugar diet.

704T **The sexually dimorphic role of octopamine receptors in gut-brain communication** Emily A Gagliano[1], Ashley Bielawski[2], Solange Holman[3], Sarah J Certel[1] [1]Neuroscience, University of Montana, [2]University of Michigan, [3]University of Montana

In order for an organism to execute a behavior, it must first assess its external environment through the lens of its internal state, prioritizing its physiological needs before acting. This internal state is driven by inter-organ communication, where critical signals exchanged between the gut and brain help integrate sensory information and guide decision-making. While this process is common to all organisms, regardless of sex, it is becoming increasingly apparent that fundamental differences in gut physiology and gene expression exist depending on whether the cells involved are intrinsically male or female. These sex differences influence gut-brain signaling and how behavioral choices are made. For these reasons, it is essential to understand what mechanisms regulate gut signaling in males and females.

Enteroendocrine cells (EEs), located in the intestinal epithelium, function as specialized secretory cells of the gut and release distinct neuropeptides, including orexigenic neuropeptide F (NPF). Previous studies indicate adrenergic receptors can influence neuropeptide release indirectly by modulating neuronal activity and neurotransmitter release. Here we examine how octopamine receptor (OAR; OA is the invertebrate analog of adrenaline) expression and function in the intestine differs between males and females by 1) quantifying sex differences in OAR transcript levels in the midgut, 2) identifying differences in neuropeptide levels upon OAR manipulation, and 3) determining the role of OARs in behavioral decision-making. Using RT-qPCR, we found that the expression levels of OA α2 and β2 receptors in EE cells are significantly higher in males compared to females. To determine a behavioral relevance to this result, we initially focused on NPF-driven food consumption. Knocking down OAα2R expression in NPF+ EE cells led to a significant increase in food intake among males, suggesting enhanced NPF signaling. Given that OA mediates the effects of food availability on aggression, we tested the fighting capabilities of males with OAα2R knockdown in NPF cells and found that they initiated aggressive behavior significantly faster than control males. Finally, we determined NPF abundance is reduced in EEs with a reduction in OAα2R. Taken together, these findings highlight the complexity of behavior regulation and the essential role of OARs in modulating potential sex-specific gut-brain signaling pathways that influence feeding and aggression.

705T ***Transketolase* interacts with *Scully* for aging-sensitive cognitive decline** Carolyne Chepkosgei, Maya Solis, Paul Sabandal, Kyung-An Han Biology, The University of Texas at El Paso

Alzheimer's Disease and Related Dementias (ADRD) are progressive neurodegenerative disorders characterized by cognitive decline and are influenced by various genetic and non-genetic risk factors, including age, sleep disturbances, and social stress. Through unbiased genetic screening, we identified a novel genetic risk factor for ADRD *Scully* (*Scu*), a multifunctional mitochondrial enzyme. The *Scu* heterozygous flies (*Scu/+*) showed decline in memory and dysfunctional inhibitory control which was augmented in an aging-dependent manner. To elucidate the pathway through which *Scu* affects cognitive function, we screened *Scu* Interacting Molecules (SIMs) and identified *Transketolase* (*Tkt*), a rate limiting enzyme in the pentose phosphate pathway (PPP) important for glycolysis, which is implicated in alcoholic dementia. Like Scu/+, *Tkt/+* mutants showed aging-dependent loss of inhibitory control. We identified the mushroom body neurons as a key neural structure for inhibitory control. To determine whether *Tkt* deficiency affects mushroom body (MB) synaptic integrity, we conducted immunostaining for synaptic molecules including the cell adhesion molecule Fasciclin 2, the postsynaptic molecule DLG and the presynaptic molecule BRP at the 4-day, 2-week, 4-week, 6-week and 8 weeks-old. Across all the ages tested, the *Tkt/+* flies showed no significant changes in Fasciclin 2 expression patterns and levels in the MB axons. We are currently assessing DLG and BRP as well as the memory capacity of *Tkt/+*. These findings will narrow the knowledge gap on how genetic risk factors interacting with aging for ADRD.

706T **Studying ageing as a two-phase process: trans-disciplinary insights from the Smurf phenotype** Flaminia Zane[1], Céline Cansell[2,2], Tristan Roget[3], Sylvie Méléard[3], Michael Rera[4] [1]Sorbonne Université, [2]INRAe, [3]Ecole Polytechnique, [4]Institut Jacques Monod, CNRS Aging is a complex, multifaceted process influenced by genetic, molecular, and environmental factors that is generally defined as a time-dependent decline of an organism's physiological functions ultimately leading to its death. In the past ten years, the breakdown of intestinal barrier function, as demonstrated in Drosophila (Rera et al., PNAS, 2012), serves as a critical indicator of systemic aging. The loss of gut integrity dubbed "Smurf phenotype" predicts hallmarks of ageing such as inflammatory responses and metabolic dysregulation better than chronological age, as well as ultimately mortality (Zane et al., Aging Cell, 2023).

Its broad evolutionary conservation across *Caenorhabditis elegans*, *Danio rerio* (Dambroise et al., Scientific Reports, 2016) and mice (Cansell et al., BMC Biology, 2023) has led us to propose a model of ageing being a two-phase process (Tricoire and Rera, PLOS ONE, 2015) and assess its role on the evolution of an "ageing function" (Roget et al., eLife, 2024).

Together, these studies illustrate the importance of considering the two-phase model of ageing for better understanding healthspan and identify potential targets for therapeutic strategies aimed at enhancing resilience against age-related diseases.

707T **Octopamine: the link between reproduction and exercise response in female *Drosophila*** Annie Backlund, Meghan Green, Emie K. Vandiver, Laura K. Reed Biological Sciences, University of Alabama

Regular physical exercise has been shown to improve physical and psychological well-being through a variety of mechanisms, such as improving insulin sensitivity and reducing blood pressure and inflammation. However, the degree to which different individuals respond to exercise varies, with sex and genetics also playing large roles in this variation. *Drosophila* has been used as a model organism to further understand the molecular mechanisms that underlie exercise adaptation. *Drosophila* can be exercised using the Power Tower, a device which subjects the flies to a repetitive motion which drops them to the bottom of their vial, taking advantage of the flies' negative geotaxis. Previous studies have found that male flies can improve their endurance, flight performance, climbing speed, and cardiovascular health after three weeks of exercise training on the Power Tower, while females are unable to gain these positive adaptations. Essential for flies' ability to adapt to exercise, octopamine is a hormone and neurotransmitter found in invertebrates that is analogous to norepinephrine. Furthermore, differences in octopaminergic neuron activity have been implicated in the sexual dimorphism in exercise response. Interestingly, octopamine is also crucial for female post mating responses, and no studies to date have investigated the interaction between exercise response and reproductive state in females. Exercise and reproduction are energetically costly processes, requiring global changes in metabolism and gene expression. Octopamine is a known metabolic regulator, so we propose that octopamine mediates the shift in metabolism required for females to allocate energetic resources to either reproduction or exercise. To test this hypothesis, we will exercise virgin and mated female *Drosophila* on the Power Tower, then measure gene expression and glucose and triglyceride concentrations. We hypothesize that mated females will be unable to acquire positive adaptations to exercise because they do not have adequate energy stores due to the energetic demands of ovulation and reproduction, and that octopamine is an important regulator of this process.

708T **Interrogating the Role of Mitochondria in Thermoregulation/Adaptation in *Drosophila*** Snigdha Gupta, Hong Xu National Heart, Lung, and Blood Institute

Temperature is a key abiotic factor affecting ecology, evolution and maintenance of species. The ability to tolerate cold is a determining factor in survival and distribution in ectotherms. The tolerance of cold stress is a result of various adaptation strategies, among others the mitochondria are an important player. Mitochondrial metabolism affects cellular bioenergetics and redox balance making these organelles an important determinant of organismal performances such as growth, locomotion, or development. Shifts in temperature can alter metabolic rates causing rearrangement of specific pathways elevating the relative cost of the mitochondrial maintenance consequently affecting the organismal fitness and adaptations. Chill coma recovery time (CCRT) is a common method to study cold tolerance in chill-susceptible insects like Drosophila. This study aims to investigate the role of seemingly non-essential mitochondrial genes in thermoregulation/ adaptation in ectotherms. Firstly, the fly mitocarta (inventory of 986 fly mitochondrial genes) was generated and the genes were grouped into different categories (metabolic, transporter, import machinery, development/signaling and unknown) based on their functionalities. Screening for non-essential genes was conducted by knocking down each of the mitocarta gene using a ubiquitous GAL4 driver and investigate survival, fertility and climbing assay. A list of 310 non-essential mitochondrial genes has been generated. Further, CCRT assay was conducted on the non-essential genes analyze if they respond to temperature changes/chill coma. This is an ongoing process but till now we observed seven mitochondrial genes (CG4120 knockdown (kd) flies being the most impacted) have impaired CCRT being cold tolerant in comparison to wildtype flies. We also made a very interesting observation where ubiquitous knockdown of almost some mitochondrial genes did not respond to chill coma and were active even after the cold shock (CG14757 kd flies being the most active). Locomotor activity was performed for the above-mentioned genes to validate the observations. Further, investigating mechanisms, would not only help identify mitochondrial thermosensors but also providing deeper insights into unexplored dimensions of mitochondrial functionality.

709T **Mechanistic Characterization of Nephrotic Syndrome Using a *Drosophila* Model** Ying Liu[1], Ping Kang[2], Ezequiel Dantas[3], Marcus D Goncalves[3], Hua Bai[2], Norbert Perrimon[1] [1]Harvard Medical School, [2]Iowa State University, [3]New York University Grossman School of Medicine

Nephrotic syndrome is frequently observed across a range of diseases and physiological conditions, including cancer, diabetes mellitus, medication effects, and aging. Despite many shared symptoms across these conditions, the existence of a common pathological mechanism underlying nephrotic syndrome remains unknown.

Using a Drosophila gut tumor model, we investigated the etiology of nephrotic syndrome. We demonstrate that Pvf1, a ligand of the Platelet-derived Growth Factor (PDGF)/Vascular Endothelial Growth Factor (VEGF) signaling pathway, is secreted by gut tumors and targets downstream pathway in the Malpighian Tubules (MT), the fly's renal system. This activation alters gene expression in the MT, resulting in phenotypes that closely mimic paraneoplastic nephrotic syndrome. Although paraneoplastic nephrotic syndrome is traditionally attributed to the side effects of cancer therapies, our research indicates a novel mechanism whereby tumor-secreted factors may directly contribute to this syndrome.

Interestingly, we observed that aging independently induces similar renal dysfunctions, suggesting a convergent mechanism with tumor-induced effects on the MT. Notably, reversing gene misexpression in the MT significantly improved the flies' longevity. These findings indicate that aging, much like tumorigenesis, may drive nephrotic changes through targeting renal gene expression, ultimately contributing to mortality.

Our study uncovers a novel mechanism by which gut tumors and aging impair renal function, highlighting potential clinical implications for managing both cancer- and aging-related nephrotic syndrome.

710F **Gene-by-Environment Analysis of Sleep Deprivation on Dietary Choice and Water Consumption in *Drosophila*** Jhilam Dasgupta, Megan Lawson, Allison John, Katie Traeger, Laura K Reed The University of Alabama

Sleep deprivation can dramatically impact appetite, mood, and can cause major health issues if left unaddressed. This includes metabolic syndrome, a risk factor for diseases like diabetes and high blood pressure, that is increasing at alarming rates worldwide. Sleep varies between individuals, and it is unclear how these variations impact metabolic health. To study these effects, *Drosophila* Genetics Reference Panel (DGRP) natural sleeping phenotypic strains that exhibit little sleep (short-sleeping), average sleep (normal-sleeping), and abundant sleep (long-sleeping) were placed in a *Drosophila* sleep deprivation apparatus, which shakes the flies for five seconds in random one-minute intervals during the night over a four-day period. Activity was monitored to compare daytime and nighttime activity between short-sleeping, normal-sleeping, and long-sleeping DGRP strains. Immediately after the sleep deprivation period, a choice capillary feeding (CaFe) assay was performed to measure consumption of high sugar, normal sugar, and high fat (multichain triglyceride oil) diets. We have chosen these diets to better represent the most common westernized diets that are correlated with reduced metabolic health. We further tested to see if there is a degree of dehydration in sleep deprived strains compared to control strains by adding a capillary tube with water to our CaFe Assays. This experiment seeks to identify the extent to which sleep deprivation affects dietary choice and water consumption in different sleep strains. We hypothesize that all sleep-deprived strains will have increased food and water intake compared to strains that were not sleep-deprived. Furthermore, we predict the sleep deprived short and long-sleeping strains will consume more of the high sugar diet to compensate for energy, as well as consume more water to compensate for dehydration. In the future, by replicating these experiments across multiple DGRP lines, we will be able to identify genetic factors that may increase or decrease diabetic risk factors due to sleep deprivation. These results will be useful in determining the relationship between sleep and metabolic health in humans.

711F **cAMP-mediated signaling in *Drosophila melanogaster*: a key regulator of muscle integrity and a potential therapeutic target for muscle atrophy** Felipe Berti Valer, Marylu Mardegan de Lima, Luciane Carla Alberici Department of BioMolecular Sciences, University of São Paulo

Muscle atrophy, considered by WHO as an urgent global health issue, is a debilitating condition, still lacking effective treatment, characterized by the loss of muscle mass and function, often linked to chronic diseases and prolonged disuse. The onset of atrophy is a complex process resulting from increased protein degradation (proteolysis) through signaling pathways such as the ubiquitin-proteasome system (UPS) and the autophagic-lysosomal pathway, and/or by the reduced rate of protein synthesis, primarily regulated by the insulin pathway. Mitochondrial dysfunctions, characterized by dynamic imbalance and mitophagy disruption, often associated with increased ROS generation, can also induce muscle atrophy. Several studies from our group suggest that cyclic adenosine monophosphate (cAMP) modulation positively impacts muscle mass preservation, neuromuscular transmission, and mitochondrial function in rodents. cAMP signaling in skeletal muscle promotes anabolic protein metabolism and attenuates the expression of ubiquitin ligases and the activity of the UPS under both basal and atrophic conditions. However, to our knowledge, no studies have demonstrated the importance of cAMP-mediated signaling for the muscle integrity of *Drosophila melanogaster*. Thus, we aimed to characterize the role of cAMP in maintaining muscle mass and function in *D. melanogaster* and its effects on mitochondrial metabolism. Using the *UAS-Gal4* system, we modulated the mRNA levels of *rutabaga* (*rut*), which encodes adenylyl cyclase ortholog, responsible for cAMP formation. At 25 °C, *rut* knockdown in muscle (*Mef2-Gal4>rut-RNAi*) resulted in lethality of all animals during the pupal stage. At 18 °C, surviving adults showed reduced fitness, shorter lifespan, impaired climbing ability, and muscle atrophy in both L3 larvae and adults. When fasted in 10% sucrose for 5 and 10 days, they lost protein mass faster than controls and showed compromised mitochondrial function with lower respiratory capacity. Ectopic expression of *rut-RNAi* in the eye using *GMR-Gal4* also reduces the size of the ommatidia. However, this phenotype was completely rescued by expressing the downstream transcription factor activated by cAMP signaling *UAS-CrebA* in this genetic background. Our results suggest that *rut* and its product cAMP are essentials for the formation and maintenance of muscle integrity in *D. melanogaster*, and position cAMP as a promising therapeutic target for muscle atrophy.

712F **Impaired Glycolysis and Bioenergetic Reprogramming in Models of Tauopathy and Frontotemporal Dementia** Anwar Nakhla, Christina Mansour, Yan Tong, Rushi Makadia, Ho Hang Leung, Ching-On Wong Biology, Rutgers University Newark

Glucose hypometabolism in the brain is a common feature in multiple neurodegenerative disorders. While glucose serves as the primary fuel for cellular bioenergetics, the role of dysregulated glycolysis in disease pathogenesis and progression remains unclear. Here, we interrogate the role of glycolysis in fly models of tauopathy and frontotemporal dementia (FTD). Neuronal overexpression of human *MAPT* in flies led to reduced expression of glycolytic genes and coincided with mitochondrial oxidative stress in neurons. Notably, genetically enhancing glycolysis in MAPT-overexpressing neurons improved both lifespan and locomotor activity in these flies, suggesting that impaired glycolysis may drive disease processes in tauopathy. Indeed, promoting glycolysis also rescued lifespan and locomotor deficits in a fly model of FTD, which expressed the $MAPT^{P301L}$ variant. Live-neuron imaging further revealed that FTD neurons exhibit reduced ATP production from glycolysis and an increased reliance on oxidative phosphorylation. Thus, tauopathy disrupts glycolysis and shifts the bioenergetic balance in neurons. Our findings highlight the potential of restoring bioenergetic homeostasis as a therapeutic strategy in neurodegenerative diseases.

713F **A nutrient-responsive Hugin-PK2 hormonal circuit regulates AKH secretion and metabolic homeostasis in female *Drosophila*** Usama Saeed[1], Takashi Koyama[2], Kenneth Veland Halberg[2] [1]Biology, University of Copenhagen, [2]University of Copenhagen

Abstract

Hormones orchestrate virtually all physiological processes in animals, enabling them to adjust internal responses to meet diverse physiological demands. In humans, as in insects, the regulation of insulin and glucagon is a very fine-tuned mechanism. In *Drosophila melanogaster*, adipokinetic (AKH), and juvenile hormone (JH) are vital for energy homeostasis, development and other physiological pathways respectively.

However, the factors regulating AKH and JH release from the corpora cardiaca (CC) and corpora allata (CA) remain virtually unexplored. The aim of this study was to investigate the role of the neuropeptides Hugin/PK2 homologs of mammalian neuromedin U, in regulating AKH and JH secretion.

To gain insight into the physiological actions of AKH-producing cells and potential regulators of AKH secretion, we identified novel receptor genes that are highly enriched in the CC. Both in males and females, FPKM values were consistently high for some genes that have been reported to regulate AKH secretion, such as *PK2-R2*, *Dh44-R2* and *CapaR*. Here, I show that spatially and temporally regulated RNAi mediated *PK2-R1* and *R2* gene silencing extends both starvation and desiccation resistance. These data suggest that Hugin/PK2 signaling regulates AKH secretion from the CC.

Furthermore, immunocytochemical approaches show that *PK2-R1*, *R2* are both expressed in the CC and in the CA, while a subpopulation of Hugin/PK2 positive neurons in the brain descend towards the CC and CA as well. Genetic ablation of hug neurons and CA resulted in reduced fecundity, but it has no effect on hatchability in nutrient deficient condition suggesting hug^{+} neurons are potentially maintaining energy and reproductive success. Knocking down of hug/PK2 receptors disrupted AKH secretion from the CC, during nutrient-deficient conditions and led to reduced reproductive success.

Additionally, knock down the hug/PK2 receptors in the CA critically impacted reproduction during nutrient stress indicated that hug is responsible for regulation of JH from CA. We have also tested hug/PK2 neurons are sensitive to internal signal related to nutrients, and we found very interesting results as we expected they are activated while we put animals on starvation media that support our whole hypothesis.

Together, the data presented in this study strongly suggests the role of Hugin/PK2 signaling in directly regulating AKH and JH secretion from the CC and CA and both hormones was discovered to be essential for reproductive success during caloric restrictive conditions. Beyond addressing fundamental questions in endocrinology and sugar metabolism, this project may unmask potential therapeutic targets for the treatment of human endocrine disorders such as diabetes and obesity.

Keywords: hug/PK2, AKH, JH, *Drosophila melanogaster*, reproductive stress, energy homeostasis

714F **Mitochondrial alternative oxidase-driven accelerated growth and development in *Drosophila melanogaster*** Marcos T Oliveira, Murilo F Othonicar, Geovana S Garcia Biotechnology, Universidade Estadual Paulista

The alternative oxidase (AOX) is a naturally occurring enzyme in the mitochondrial electron transfer system (ETS) of most organisms, but absent in insects and vertebrates. It oxidizes reduced coenzyme Q and reduces O_2 in H_2O, partially replacing the ETS cytochrome c segment and alleviating the oxidative stress caused by ETS overload. Successfully demonstrated in animal models, AOX shows potential in mitigating mitochondrial diseases. However, its non-proton-pumping nature might uncouple mitochondria, leading to excessive heat generation and interference with normal metabolism and physiology. In fact, we have previously shown that AOX from *Ciona intestinalis* enhances resistance to cold stress in *Drosophila melanogaster*. To investigate the underlying uncoupling mechanisms and metabolic impacts of AOX, we examined diverse phenotypes of AOX-expressing *Drosophila* larvae at 25°C and at the stressful temperature of 12°C, along with mitochondrial physiology, ETS composition and general metabolism by high-resolution respirometry, blue native-polyacrylamide gel electrophoresis, target metabolomics and fluorescence lifetime imaging microscopy. Our findings indicate that AOX larvae display up to 70% increased locomotor activity and elevated heat production, maintaining body temperature ~0.2°C higher than controls. Additionally, they have ~33% more dry mass, primarily due to elevated fat accumulation and a twofold increase in the rate of food intake. Detailed mitochondrial analyses reveal that AOX intensifies Leak respiration and lowers oxidative phosphorylation efficiency through functional interactions with the mitochondrial glycerol-3-phosphate dehydrogenase (mGPDH). This is associated with increased complex I (CI)-driven respiration, which appears to occur via increased supercomplex formation, and with an enhanced metabolic flux through the tricarboxylic acid (TCA) cycle, as judged by significantly lower NADH/NAD+ ratio and TCA cycle intermediates, like citrate and malate. Thus, AOX appears to be reinforcing the larval metabolic program, which is highly proliferative, like human tumors. We present here an intricate mechanism in which AOX uncouples preferentially mGPDH-driven respiration and amplifies CI-driven cataplerosis, promoting thermogenesis and fostering larval growth, particularly under severe cold stress.

715F **Polyunsaturated fatty acids stimulate immunity and eicosanoid production in *Drosophila melanogaster*** Pakeeza Azizpor[1], Ogadinma Okakpu[2], Sophia C Parks[2], Diego Chavez[2], Fayez Eyabi[2], Adler R Dillman[1] [1]Nematology, University of California, Riverside, [2]University of California, Riverside

Eicosanoids are a class of molecules derived from twenty carbon (C20) polyunsaturated fatty acids (PUFAs) that play a vital role in mammalian and insect biological systems, including development, reproduction, and immunity. Recent research has shown that insects have significant but lower levels of C20 PUFAs in circulation in comparison to eighteen carbon (C18) PUFAs. It has been previously hypothesized in insects that eicosanoids are synthesized from C18 precursors, such as linoleic acid (LA), to produce downstream eicosanoids. In this study, we show that introduction of arachidonic acid (AA) stimulates production of cyclooxygenase, lipoxygenase, and cytochrome P450–derived eicosanoids in *Drosophila melanogaster*. Downstream immune readouts showed that LA stimulates phagocytosis by hemocytes, while both LA and AA stimulate increased antimicrobial peptide expression when *D. melanogaster* is exposed to a heat-killed bacterial pathogen. In totality, this work identifies PUFAs that are involved in insect immunity and adds evidence to the notion that *Drosophila* utilizes immunostimulatory lipid signaling to mitigate bacterial infections. Our understanding of immune signaling in the fly and its analogies to mammalian systems will increase the power and value of *Drosophila* as a model organism in immune studies.

716F **Non-olfactory expression of olfactory receptors in *Drosophila*** Yumiko Ukita[1], Ryoka Suzuki[1], Keita Miyoshi[2], Tzu-Chiao Lu[3], Kuniaki Saito[2], Hongjie Li[3], Misako Okumura[1], Takahiro Chihara[1] [1]Graduate School of Integrated Sciences for Life, Hiroshima University, Japan, [2]Invertebrate Genetics Laboratory, National Institute of Genetics, Japan, [3]Huffington Center on Aging & Department of Molecular and Human Genetics Baylor College of Medicine, USA

Olfaction plays a crucial role to detect environmental odorants derived from food, mating partners, pathogens and so on. These odorants are detected by olfactory receptors exclusively expressed in the dendrite of olfactory receptor neurons. In addition, the expression of olfactory receptors in non-olfactory tissues has been reported in vertebrate such as mice, zebrafish and humans. However, it is largely unclear whether invertebrate olfactory receptors are expressed in non-olfactory tissues.

Here, we show that *Drosophila* odorant receptors (Ors) are expressed in non-olfactory tissues at single-cell resolution by using Fly Cell Atlas (FCA), single nucleus RNA-seq data of the entire adult *Drosophila* which cover 251 cell types in the whole body. Some *Drosophila Ors* are highly expressed in non-olfactory tissues as well as sensory neurons. To confirm the non-olfactory expression of Ors *in vivo*, we examined the expression of *Or-Gal4* drivers in non-olfactory tissues; however, most *Or-Gal4* drivers could not label the non-olfactory cells. To precisely monitor the endogenous expression of *Or* genes, we generated a series of *T2A-QF2* knock-in lines for 21 *Or* genes. In these lines, *T2A-QF2* is inserted before the stop codon of *Or* genes and the expression of QF2 should reflect the endogenous expression of *Or* gene. Using these genetic tools, we found that some *Or* genes are indeed expressed in the non-olfactory tissues, including the male accessory gland, consistent with the FCA data. Male accessory gland secretes seminal fluid proteins which are transferred to female during mating, leading to the success of fertility. We will discuss the function of Ors in non-olfactory tissues, particularly in the male accessory gland.

717F **Sex differences in the *Drosophila* larval fat body** Celena M. Cherian, Elizabeth J. Rideout Cellular and Physiological Sciences, University of British Columbia

The *Drosophila* larval fat body is an endocrine organ that produces and secretes peptides that influence development and physiology. Previous work from our lab established that the sexual identity of the larval fat body plays a key role in promoting a larger body size in female larvae. Yet, how sex differences in the larval fat body influence body size is not understood. Our analysis of larval fat body gene expression revealed significant sex differences in gene expression in this organ. In particular, females had significantly higher mRNA levels of genes that encode ribosomal and mitochondrial proteins. These differences in gene expression are significant, as we found that female fat body cells have more nascent protein synthesis and markers associated with higher mitochondrial activity. To identify regulatory factors that contribute to sex differences in protein synthesis and mitochondrial activity we explored a role for metabolic regulator *spargel* (*srl*), as studies in mixed-sex groups show that *srl* regulates an array of genes involved in mitochondrial function and ribosome biogenesis. In female larvae with heterozygous loss of *srl*, there was a significant decrease in protein synthesis and mitochondrial activity with no effect on these phenotypes in male larvae. Overall, my data suggests that female larval fat body cells have higher levels of protein synthesis and mitochondrial activity and that this is regulated by *srl*.

718F **Elucidating the Role of Mitochondrial Pyrophosphatase in Sudden Cardiac Death Using Drosophila Models** Min Li, Fan Zhang, Hong Xu National Heart Lung Blood Institute

Sudden cardiac death (SCD) accounts for 15-20% of all deaths, with 180,000 to 450,000 cases in the US annually. Mitochondrial DNA (mtDNA) plays a key role in the onset and progression of SCD. Recent studies have identified mutations in the PPA2 gene, encoding mitochondrial inorganic pyrophosphatase, associated with severe cardiac outcomes and SCD, particularly in adolescents where alcohol consumption can trigger fatal events. However, the precise mechanism by which PPA2 dysfunction leads to cardiac arrhythmias and sudden death remains poorly understood. Animal models are needed to explore the mechanisms linking mitochondrial pyrophosphatase deficiency to SCD. With its *nurf38* gene homologous to human PPA2, *Drosophila melanogaster* provides an excellent model system for this research. This project aims to elucidate the molecular mechanisms by which mitochondrial pyrophosphatase dysfunction contributes to cardiac arrhythmias and sudden death. The hypothesis behind the proposed work is that deficiency of mitochondrial pyrophosphatase results in pyrophosphate (PPi) accumulation, impairing both mitochondrial DNA maintenance and gene expression, leading to mitochondrial dysfunction, reducing heart rate reserve and delaying heart rate recovery, ultimately causing cardiac dysfunction and sudden cardiac death. To address this hypothesis, the research design involves genetic engineering of Drosophila, comprehensive cardiac phenotyping, and molecular analysis. We will generate *Drosophila* models with *nurf38* mutants using genetic engineering techniques to mimic human PPA2 mutations and comprehensively assess the cardiac function using optical, electrophysiological, and histological methods to understand how mitochondrial pyrophosphatase dysfunction impacts heart performance and structure. We will explore the molecular mechanisms by which *nurf38* loss leads to mitochondria dysfunction, testing the hypothesis that PPi accumulation leads to defective mtDNA maintenance and gene expression. We will mimic human SCD triggers and determine whether the heart rate reserve and recovery are reduced in the *nurf38* mutants by challenging the flies with cardiac pacing and environmental stressors. The proposed work will yield powerful genetic models of inorganic pyrophosphatase deficiency to enable the study of the function of pyrophosphate in the heart, and also significantly advance the understanding of how PPA2 mutations contribute to sudden cardiac death, which will pave the way for developing novel therapeutic approaches to reducing the incidence of SCD.

720F **Investigating a developmentally crucial adipose tissue-derived factor in regulating nutritional homeostasis in *Drosophila melanogaster*** Anindita Rao, Jishy Varghese School of Biology, Indian Institute of Science Education and Research, Thiruvananthapuram, Kerala

Organisms employ a range of physiological and biochemical strategies to maintain nutrient balance when faced with environmental stressors—an essential process for survival and optimal function. These strategies involve diverse adaptive responses. The adipose tissue poses as a pivotal element in the organism's ability to mitigate such environmental fluctuations. We aim to identify crucial regulatory factors, that govern nutrient equilibrium, within the fat body using a targeted genetic screening approach.

Our approach led to the identification of Larval Serum Proteins (LSPs), a member of the hexamerin family of storage proteins, as a key player in nutritional homeostasis. The *Drosophila* genome contains two hexamerin genes, *Lsp1* and *Lsp2*, which are predominantly expressed in the larval fat body. These proteins function as amino acid reservoirs in insects, particularly during metamorphosis, facilitating adult emergence. While much of the research on hexamerins has been from a biochemical perspective, functional studies using genetic and molecular techniques remain limited. In our study, we specifically knockdown LSPs in the larval fat body to examine its effects on development and metabolic status.

Contrary to our expectations, reducing Lsp1 in the developing larval fat body results in increased organismal growth and an energy imbalance. Lsp1 also appears to play a role in responding to low nutrient conditions, and its downregulation triggers growth via TOR signaling activation. Additionally, Lsp1-deficient flies exhibit heightened survival rates under acute starvation, demonstrating resilience during dietary scarcity. Intriguingly, the outcome of silencing LSP1 varies between male and female flies, impacting their feeding responses and lifespans in distinct ways. Current investigations are focused on evaluating potential trade-offs in reproductive fitness and stress susceptibility. Our goal is to uncover the regulatory mechanisms by which hexamerins help manage alterations in the nutritional environment.

We will discuss the unexpected results of reduced Drosophila LSP expression, which were beneficial to the organism than negatively affecting adult maturation. This research will provide novel insights into hexamerin-mediated maintenance of nutrient homeostasis.

721F **Maternal Diet Shapes Embryo Development and Offspring Phenotype in *Drosophila melanogaster*** Krittika Sudhakar[1], Zachary Madaj[2], Adelheid Lempradl[1] [1]Department of Nutrition and Metabolism, Van Andel Institute, [2]Bioinformatics and Biostatistics Core, Van Andel Institute

Parental diet significantly impacts offspring development, with prenatal factors like maternal nutrition and stress influencing adult offspring phenotypes. This concept aligns with the Developmental Origins of Health and Disease (DOHaD) hypothesis, which posits that maternal conditions during pregnancy can shape offspring's health and development. Intriguingly, in *Drosophila melanogaster*, where eggs develop independently of the mother after laying, these effects suggest a pre-encoded environmental influence within the egg. Our study investigates this phenomenon by exposing female flies to low-, medium-, and high-sugar diets for 10 days before mating with males on a control diet. We examined body weight, lifespan, fecundity, and triglyceride (TAG) levels in both mothers and their F1 offspring. Notably, a low-sugar maternal diet shortened lifespan, whereas a high-sugar diet increased maternal TAG levels. In the F1 generation, offspring from high-glucose-fed mothers experienced a ~2-hour delay in hatching time. Furthermore, female offspring from both low- and high-sugar diets displayed enhanced starvation resistance compared to medium-sugar controls. To explore the underlying mechanisms, we utilized single-embryo RNA sequencing and metabolomics techniques developed in our lab. One of the frequent hits was the Kynurenine (Kyn) pathway, and the approaches we use to test the role of Kyn are via supplementation and mutants. We intend to use single-cell sequencing techniques to explore the underlying process's details further. Our findings highlight that maternal nutrition imposes lasting metabolic challenges on offspring, resulting in phenotypic changes, and support the DOHaD hypothesis even in species like *Drosophila*, where maternal influence ceases post-laying.

722F **ERR and HSF cooperatively regulate cellular metabolism** Yuan Feng[1], Sunandan Chakrabarti[1], Chunyang Zhang[2], Brian Calvi[2], Richard Carpenter[1], Jason Tennessen[2] [1]School of Medicine, Indiana University, [2]Department of Biology, Indiana University

Metabolism must be precisely regulated to provide appropriate energy and building blocks for cellular homeostasis, growth, and survival. Inappropriate alterations in metabolic flux underlie a wide range of human diseases and even represent a hallmark feature of cancer cells. In this regard, one of the most notable metabolic alterations observed in cancer cells is known as the Warburg effect (aerobic glycolysis), which is characterized by elevated levels of glycolytic flux and aerobic lactate production. The resulting glycolytic program is suited to rapidly metabolize carbohydrates for biomass production in tumors, thus supporting cell growth and proliferation. While aerobic glycolysis is commonly associated with cancer cells, this form of glycolytic metabolism can also promote normal growth and development. In *Drosophila melanogaster*, aerobic glycolysis is activated prior to the onset of larval development, thus promoting a glycolytic state that supports rapid larval growth. This observation establishes the fly as an ideal model for studying the endogenous molecular mechanisms that regulate the Warburg effect. Previous studies demonstrated that the *Drosophila* Estrogen-Related Receptor (dERR), an orphan nuclear receptor, is a key activator of aerobic glycolysis. In *dERR* mutants, late-stage embryos fail to activate aerobic glycolysis, rendering larvae unable to use dietary carbohydrates to support biomass accumulation. While the role of dERR in regulating the larval glycolytic program is well-documented, the mechanisms that control dERR activity during this time remain poorly understood. During our ongoing studies of dERR regulation, we found that heat shock causes dERR to translocate from the cytoplasm into the nucleus in the somatic follicle cells of the ovary. This result raises the possibility that ERR activity is coordinated with the cellular heat response, and implicates heat shock transcription factor (HSF), which is the master regulator of cellular heat response, in mediating environmental regulation of dERR activity and cellular metabolism. Consistent with this idea, our multi-omics data suggested that mutations in the *Drosophila* ortholog of the HSFs (*dHsf*) result in larval lethality and induce defects in glycolytic processes and gene expression, similar to those observed in *dERR* mutants. Moving forward, our goal is to identify the molecular mechanisms by which dHsf regulates dERR activity in the context of glycolytic metabolism. By leveraging the special insights offered by *Drosophila*, our long-term aim is to not only identify the conserved mechanisms linking cellular stress response, metabolic regulation, and developmental growth but also reveal new metabolic vulnerabilities within cancer cells.

723F **Characterization of visual phenotypes of insulin receptor hypomorphs in *Drosophila melanogaster*** Luis Fernando Medina-Perez, Juan Riesgo-Escovar Instituto de fisiología celular, Universidad Nacional Autónoma de México

Mammalian diabetic retinopathy is a severe ocular complication caused by chronic high blood glucose levels, leading to retinal damage. To better understand the retinal effects associated with diabetes, this study employs insulin receptor (*InR*) hypomorphs in *Drosophila melanogaster* as a model for exploring early retinal complications. Utilizing heteroallelic mutants (InR^{3T5} and InR^{E19}) for the insulin receptor gene, we aim to characterize behavioral, physiological, and anatomical changes in the retina. To accomplish this, we performed behavioral tests and electroretinograms at different ages to assess alterations in the visual system of the fly. Our results shows that insulin receptor mutants exhibit impaired visual responses in behavioral tests and a reduced response to light in electroretinograms compared to control groups, reflecting the impact of reduced insulin signaling pathway. This research provides important insights into the development of diabetic retinopathy and early retinal consequences of impaired insulin signaling, contributing to the broader understanding of diabetic retinopathy mechanisms, as flies have an open circulatory system, and thus, allows us to distinguish between non-vascular (this work) and vascular complications in visual tissue.

724F **Gene network-based approaches to understand aging and lifespan from *D. melanogaster*** Savandara L Besse, Michael Rera CNRS, Institut Jacques Monod

Aging is commonly described as the time-dependent progressive dysfunction of biological processes, referred to as the hallmarks of aging. Michael Rera and his team have developed a theoretical framework and shed new light on aging biology. Contrary to the classical view, this model describes aging as having two consecutive phases, each with distinct molecular and physiological properties in the fruit fly *D. melanogaster* (Tricoire and Rera, 2015). The transition is detected in vivo using a non-toxic and non-absorbable blue food dye that the intestine becomes permeable to, before the individual›s natural death. At any moment, a given population comprises two subpopulations, the “healthy” non-Smurfs and the end-of-life Smurfs. In particular, using bulk transcriptomics data from Smurf individuals (the 2nd phase of aging or the end-of-life phase), his team has shown stereotypical transcriptomic signatures associated with known transcriptional hallmarks of aging (Zane et al., 2023) as being restricted to the Smurf phase, independently of individuals› chronological age. Building upon these recent results, using multi-omics integrative strategies relying on biological networks - known or inferred - we are now able to develop a better understanding of aging›s complexity. We will present different gene networks that help propose new hypotheses on the drivers of aging.

726F **Exploring the impact of antioxidant-rich diets on healthspan using *Drosophila melanogaster*** Moumita Chakraborty, Mark A Phillips Oregon State University

Antioxidants have been studied for over a decade due to their reported benefits with respect to increasing longevity and overall health span. Using the Drosophila model system, researchers have also worked extensively to understand the strength of these effects and the mechanisms that underlie them. However, these studies are typically down in isogenic lines and are not representative of the variation we observe in natural populations. In this study, we aim to test the effects of two antioxidant diet supplements, apple and pomegranate, on large outbred *Drosophila melanogaster* population. In doing this, we hope to get a more accurate estimation of their general efficacy and how their effects vary with genetic background. Specifically, we measure lifespan, reproductive output, and neurological function via climbing assays to address the impacts of these supplements on on the health span. Initial results from four replicates showed that the mean lifespan of male flies fed pomegranate supplement (PmM) was extended by 3.15%, whereas, the apple-fed male flies (ApM) slightly decreased compared to the control treatment. Overall, male flies depicted higher lifespan among all treatments compared to male and female flies. We are continuing the mortality and fecundity assay in four new replicates, which will give us a complete estimate. Using metabolomic and transcriptomic data, we also hope to characterize the mechanisms underlying their effect and quantify how they impact “biological age”.

727F **Lipase mediated gut-brain communication regulates insulin secretion in Drosophila** Abhilasha Kandahalli Venkataranga Nayaka[1], Alka Singh[2], Jairaj K Acharya[1], Usha Acharya[1] [1]Cancer & Developmental Biology Laboratory, National Cancer Institute, [2]Department of Molecular, Cell and Cancer Biology, UMass Chan Medical School

Pancreatic β cells synthesize and secrete insulin in response to elevated glucose to maintain blood glucose levels within the physiological range. Defective insulin secretion is causal in almost all forms of diabetes. While glucose is the primary stimulus for insulin secretion by the b cells, it is known that lipids obtained from diet or generated intracellularly can augment the glucose-stimulated insulin secretion (GSIS). Further, other organs differentially regulate insulin secretion in response to various diets via inter-organ communication. The insulin pathway is also conserved in Drosophila and insulin-like peptides (ILPs) are synthesized and secreted from neurosecretory cells called insulin producing cells (IPCs) in the brain. We show that a Drosophila secretory lipase, Vaha (CG8093) is synthesized in the midgut and moves to the brain where it concentrates in the insulin producing cells. Vaha utilizes the lipoprotein LTP and LDL like receptor (LRP1 & LRP2) transport system to cross the Blood Brain Barrier and concentrate in IPCs. Vaha stimulates insulin-like peptide release in response to dietary fat since loss of Vaha results in reduced insulin-like peptide secretion and diabetic features including hyperglycemia and hyperlipidemia. Constitutive activation of IPCs rescues Vaha mutant phenotypes. Additionally, loss of Vaha does not affect glucose metabolism in ILP mutants. These findings suggest Vaha lipase influences glucose metabolism via insulin. Furthermore, our results show Vaha functions as a diacylglycerol lipase physiologically serving as a molecular link between dietary fat and lipid amplified GSIS in a gut-brain axis.

728F **Effects of exercise on starvation-selected *Drosophila melanogaster*** Katrina Pinili, Elena De La Torre, Allen G Gibbs University of Nevada-Las Vegas

We studied the effects of exercise on replicated populations of *Drosophila melanogaster* that have been selected for starvation resistance for over 180 generations. Starvation-selected (SS) flies display cardiomyopathy, poor locomotor ability, and reduced flight ability compared to fed control (FC) flies. Because *D. melanogaster* is considered a model of both obesity and exercise, we investigated the effect of exercise on survival, locomotor ability, and endurance of SS and FC flies. Flies followed a 3-week exercise regimen using the Fly Roller, a custom-built device that rotates cohorts of flies in vials, inducing movement through their negative geotactic and positive phototactic responses. Upon exercise completion, locomotor ability was assessed by conducting a rapid iterative negative geotaxis (RING) assay. To quantify survival and endurance of flies we counted the number of survivors daily throughout the regimen, and assessed fly exercise activity from video recordings, respectively. Results suggest that exercise can increase survivability in FC and SS females but not in males. Exercise may not affect locomotor ability for flies, which decreased for all populations throughout the regimen. SS males exercised longer on the Fly Roller than females. This work highlights the impact of exercise on important life traits of obese *Drosophila*.

729F **Just Fly… Unless You Broke Your Ankle2!** Miranda Dietze, Shawn Primavera, Nichole Link Neurobiology, University of Utah

Ankle2 is a key neurodevelopmental protein that regulates neuronal stem cell asymmetric division by interacting with Ballchen/VRK1. In addition, Ankle2 is a hypothesized structural protein necessary for nuclear envelope morphology and contains a LAP2, emerin, MAN1 (LEM) domain. The LEM domain is found in nuclear envelope proteins, and mutations in these genes result in laminopathic diseases. Laminopathies span a large variety of diseases, including Progeria (rapid aging) and Emery Dreifuss Muscular Dystrophy, and often result in a range of comorbidities, including reduced patient lifespan, motor deficits, and neuropathy. Interestingly, *VRK1* mutations in humans have been linked to motor neuron diseases, which share motor and neuropathy phenotypes with laminopathies. Whether *Ankle2* loss causes a similar adult-onset phenotype has not been tested. Here, I show that *Ankle2* knockdown in *Drosophila* motor neurons results in reduced motor function and lifespan, similar to motor neuron diseases. However, the mechanisms underlying Ankle2 function in motor neurons, and whether this function requires the Ballchen/VRK1 pathway is still uncharacterized. Determining the role of Ankle2 and its downstream protein pathway in motor neuron diseases will identify a novel cause of the disease and introduce new therapeutic avenues.

730F **Amino acids exert a suppressive effect on erebosis through ammonia generation in the *Drosophila* midgut.** Yukana Nakamura[1], Motohiro Morikawa[2], Tomomi Takano[1], Sa Kan Yoo[1] [1]Laboratory for Homeodynamics, RIKEN BDR, [2]RIKEN BDR

Many adult tissues maintain homeostasis through cell turnover, which is mediated by a balance of cell death and cell proliferation. The gut is one of the tissues with the highest turnover rate. We recently discovered a new cell death form termed erebosis, which is the main cell death mechanism that occurs in enterocytes of the *Drosophila* midgut. Erebosis is characterized by flat nuclei, accumulation of angiotensin converting enzyme and loss of a variety of proteins. How external cues regulate erebosis remains unknown. Here, we investigated whether nutrients in the food that flies eat affect erebosis in the gut enterocytes. We found that ingested amino acids, irrespective of their specific types, suppress erebosis, indicating that the quantity, not the quality, of amino acids is a key regulator of erebosis. Ingestion of amino acids induced elevation of urea, uric acid and ammonia. Inhibition of urea or uric acid generation did not stop the amino acids-mediated suppression of erebosis. Addition of ammonia was sufficient to suppress erebosis, indicating that ammonia, a waste product of amino acid metabolism, is a key regulator of erebosis. This work provides the first insight into the regulatory mechanism of erebosis by the external cues.

731F **The glycolytic investment phase plays a critical role in metabolic cross-talk and nutrient utilization** Yusei Miura[1], Tomomi Takano[2], Mihoko Katayama[2], Motohiro Morikawa[2], Sa Kan Yoo[2] [1]Labratory for Homeodynamics, RIKEN BDR, [2]RIKEN BDR

While individual metabolic pathways are well-characterized, the intricate cross-talk between multiple pathways, particularly at the organismal level, remains poorly understood. In this study, we investigate the interplay between glycolysis and other metabolic processes. Glycolysis is a ten-step anaerobic pathway where the initial five steps constitute the “investment phase,” using energy to break down glucose into two molecules, while the subsequent five steps form the “payoff phase,” generating ATP. Through metabolite supplementation experiments, we found that sugars such as glucose, sucrose, fructose, galactose, mannose and trehalose, all of which enter the early investment phase, facilitated the organism’s ability to utilize other metabolites like amino acids, acetylcholine, choline and carnitine. Conversely, metabolites entering the later payoff phase (e.g., ribose, xylose, lactate and acetate) did not enhance the organism’s ability to metabolize alternative nutritional sources.

These results are surprising for two reasons: (1) endogenous sugars, despite being present within the organism, do not support the utilization of other metabolites, and (2) the non-energy-producing (investment) phase, rather than the ATP-generating (payoff) phase, plays a critical role. We will discuss mechanistic insights of these findings.

732F **Echoes of Stress: Acoustic Modulation of Ecdysteroid Signaling in *Drosophila melanogaster*** Jaelyn M Darden, Jennifer Hackney Math and Natural Sciences, Arizona State University

Environmental acoustic stress is known to modulate hormone pathways in vertebrates, yet its impact on insect steroid hormones remains understudied. In *Drosophila melanogaster*, 20-Hydroxyecdysone (20E) is a pivotal steroid hormone involved in developmental processes and is thought to mediate responses to some environmental stimuli in adult fruit flies. Here, we investigate 20E's response to acoustic stress by examining direct measurement of 20E levels, Halloween gene expression (associated with ecdysone biosynthesis), and Ecdysone Receptor activation. Preliminary results indicate acoustic stress is associated with elevated 20E titers in all treatment groups, with the most pronounced increase in virgin females. To assess its impact on ecdysteroid signaling pathways, adult flies were exposed to stressful acoustic stimuli to document tissue-specific activation patterns of Ecdysone Receptor (EcR) ligand sensors. Halloween gene transcriptional changes induced by acoustic stress were quantified using real-time PCR. These results will help us understand how sound-induced stress affects steroid-mediated physiological processes, providing a foundation for understanding acoustic stress in arthropod populations.

733F **Sex-Dependent Metabolic Shifts, Enzyme Expression, and Heart Function Alterations in *Nepl15^{KO} Drosophila*** Shahira Arzoo[1], Chase J Drucker[2], Surya J Banerjee[2] [1]Texas Tech University, [2]Biological Sciences, Texas Tech University

Obesity and type 2 diabetes (T2D) are widespread health challenges, with the annual cost of diabetes management in the U.S. reaching $414 billion in 2023. These conditions are closely linked to imbalances in metabolic homeostasis, highlighting the critical need to understand the molecular pathways involved. Neprilysin, commonly recognized for its role in amyloid-beta breakdown and blood pressure regulation, has recently been associated with metabolic functions affecting insulin sensitivity and energy regulation. However, the underlying mechanisms remain largely unexplored.

This study uses the Drosophila model to examine the role of Neprilysin-like 15 (Nepl15) in nutrient homeostasis. The Nepl15 gene shows a 4.5-fold higher expression in adult males than females, with distinct, sex-specific effects on nutrient storage. Male Nepl15 loss-of-function (LoF) mutants display markedly reduced glycogen and glycerolipid storage, while female mutants exhibit significantly elevated glycogen, despite similar food consumption levels compared to controls. Furthermore, the LoF mutants demonstrate altered expression of key metabolic enzymes, including Lipin, Glycogen Synthase, Glycogen Phosphorylase, Fatty Acid Synthase 1 and 2, and Acetyl-CoA Synthase.

Metabolomic profiling reveals sex-specific metabolite and lipid composition shifts among Nepl15 LoF adults. In females, upregulated metabolites include prunin, leucrose, and ribonic acid, while dehydroascorbic acid, glucose-6-phosphate, and citric acid are downregulated. Male mutants show increased fucose, fructose, and linoleic acid levels, with decreased levels of xylulose, dehydroascorbic acid, quinic acid, and tyrosine. As a result, females live longer, and males have an unaltered lifespan which correlates with ROS levels and ATP levels in them. This is reflected by enhanced activity levels and a stable heart function at older age in these LoF flies. Thus, the Nepl15-regulated pathway can contribute to identifying effective and robust targets to control obesity and related metabolic diseases.

734S **The effect of Centella asiatica hot water extract on neuroinflammatory responses in aging Drosophila melanogaster** Gavin C Day, Alexis A Strunz, Stanislava Chtarbanova Department of Biological Sciences, The University of Alabama

Centella asiatica is a perennial plant, extracts from which are commonly used in Eastern medicine. Being sold commercially as a health supplement, claims are made that this plant extract improves brain health and cognition in older adults. These claims have not been authenticated and the mechanism by which this supplement could work is unknown. Inflammation leads to many chronic illnesses. Our project tests the hypothesis that compared to a control regimen, treatment with a cold-water extract from Centella asiatica (CAW) lowers neuroinflammatory responses and improves neurological decline in aging Drosophila melanogaster. Newly eclosed flies separated by sex were continuously fed either 10% yeast solution or 10mg/mL CAW. The effect of CAW on lifespan as well as on inflammatory gene expression for several antimicrobial peptides (AMPs) genes targets of the Toll and IMD NF-kB pathways in 5-, 30-, 40-, and 50-day old flies were measured. CAW-treated males displayed slightly improved median lifespan in comparison to controls, while median lifespan was comparable in CAW- and control-treated females. None of the differences were statistically significant. Our preliminary data for two AMP genes, Drosomycin and DiptericinB, show a trend in 50-days old flies of both sexes, in which CAW treatment is associated with decreased AMP expression in the head. This suggests an effect of the botanical extract in older flies. Current efforts are focused on testing how CAW treatment impacts locomotor behavior with aging, as well as brain cell death. Another future direction includes testing the effects of different CAW concentrations on age-related phenotypes.

735S **Metabolic regulation during the oocyte-to-embryo transition** Misato Takagishi, Takaomi Sakai, Toshiro Aigaki, Satomi Takeo Tokyo Metropolitan University

Egg activation is the transition of mature oocytes to gain developmental competence as embryos and includes the events, Ca^{2+} oscillation, resumption of meiosis, membrane hardening, and translation of maternal mRNA. Along with these dynamic events, energy metabolism should be triggered at the time of egg activation. However, metabolic transition during egg activation remains largely unknown.

In this study, we aimed to understand how energy metabolism is regulated during egg activation and early embryonic development. To begin with, we analyzed the amounts of metabolites in wild-type mature oocytes and early embryos (0~30, 30~60, 60~90 minutes after fertilization) by using liquid chromatography mass spectrometry. Principal component analysis indicated that metabolomic profile was clearly distinct between mature oocytes and embryos.

Dramatic changes in metabolite levels were observed in glycolysis, a central energy producing pathway. The most upstream metabolite glucose increased in embryos, while some of intermediate metabolites, G6P, F6P, and FBP decreased once at the beginning, then gradually recovered. Further downstream metabolites DHAP and pyruvate decreased continuously within the observed period. We also noticed that metabolites in the TCA cycle, nucleotide precursors IMP and PRPP, and nucleoside triphosphate levels changed significantly. These results indicate that egg activation is accompanied with complex and drastic changes in glucose metabolism within a short period.

As egg activation and fertilization are independent events in *Drosophila*, we asked which were observed metabolic changes dependent on. To answer this, we performed metabolome analysis of activated eggs by crossing spermless males with wild-type females. Unfertilized eggs and fertilized embryos showed similar metabolic changes, indicating that changes in glucose metabolism were induced by egg activation and not the consequence of mitotic division after fertilization.

Glycolysis is involved in not only ATP synthesis, but also production of amino acids, nucleic acids and lipids. Since embryos undergo active synchronous nuclear divisions within about ~1.5 hours after fertilization, we hypothesized that the carbon source supplied from glycogen increased and used for nucleotide synthesis. We quantified glycogen levels and measured activity of glycogen breakdown enzyme, glycogen phosphorylase (GlyP) in mature oocytes and embryos, but neither of them changed upon fertilization. Glycogen is unlikely to be the source of glucose that is actively used at the beginning of embryogenesis.

We are currently working on phenotypic characterization of the loss-of-function mutations in glycolysis and nucleotide synthesis pathway. Our study by genetic and metabolic analyses will demonstrate the importance of regulation of energy metabolism during egg activation.

736S **Dissecting Rapamycin-sensitivity across diverse genetic backgrounds of Drosophila melanogaster** Sahiti Peddibhotla[1,2], Tony Sun[1,2], Tricia Zhang[1,1], Ricard Rodriguez-Mias[3], Benjamin R. Harrison[4], Mitchell B Lee[5], Yasha Goel[1,1], Daniel E. L. Promislow[6], Judit Villen[3], Hannele Ruohola-Baker[1,1] [1]Department of Biochemistry, University of Washington, [2]Institute of Stem Cell and Regenerative Medicine, University of Washington, [3]Department of Genome Sciences, University of Washington, [4]Department of Lab Medicine and Pathology, UW School of Medicine, [5]Ora Biomedical, [6]Human Nutrition Research Center on Aging, Tufts University

Rapamycin, an inhibitor of mTOR, has made revolutionary contributions to the field of aging. Yet, little is known about the impact of genetic variation on the response to rapamycin. In developing drugs, considering genetic variation is foundational to realizing the translational potential. In Drosophila melanogaster, rapamycin typically delays development in the larval and pupal stages. However, we have shown dramatic variation in mean pupation time in response to rapamycin across over 140 genetically diverse wild-derived inbred strains of Drosophila from the Drosophila Genetics Reference Panel (DGRP). Interestingly, this sensitivity isn't associated with genetic variation in or around the mTOR gene. Thus, we hypothesize that variation in downstream targets of mTOR accounts for the variation in rapamycin response across genetic backgrounds. Currently, we are using several approaches to understand the differences in activation of mTOR targets between highly resistant and sensitive strains when exposed to rapamycin.

Firstly we aim to uncover the cell-wide phosphoproteome of first instar larvae of Drosophila strains with extreme sensitivity and resistance phenotypes. We treated first instar larvae with rapamycin (20uM) for 12 hours, and collected samples for mass spectrometry analysis. To validate that 12 hours of treatment is sufficient to see a rapamycin response, we continued the growth of a parallel group of larvae until 72 hours and measured their size. In the sensitive DGRP strains, 348 and 517, we observe a 2 fold reduction in larvae length at 72 hours compared to control (p-value <0.0001), whereas in the resistant strain, 441, we observe no significant decrease. These data indicate that 12 hours of treatment is sufficient to elicit developmental differences. By comparing the phosphoproteome of multiple resistant and sensitive lines, we can uncover factors that highly associate with a resistant or sensitive phenotype. To understand whether sensitivity is dependent on the presence of these factors identified by mass spectrometry, we will overexpress these factors with phosphodegron mutations in w1118, our strain-control, and test for rapamycin sensitivity by measuring larval size.

Understanding the common mechanism behind resistance or sensitivity to rapamycin is critical for the drug's application in medicine. This project highlights the value of accounting for genetic variation in drug development and testing, helping to shape future approaches for developing new drugs.

737S **RNAi of the electron transport chain in glutamate neurons increases sleep, decreases locomotor activity, and extends life span** Abigail Forrest, Maria Longenecker Eastern Mennonite University

RNAi targeting the electron transport chain (ETC) has been proven to prolong life span in many different species, including within Drosophila melanogaster. In previous studies, when RNAi was activated against genes of Complex I and V in glutamate neurons, life span was extended between 18 - 24% regardless of using the D42- or VGlut-GAL4 driver lines. Additional results show limiting GAL4 activity to non-VGlut-GAL4 glutamate neurons in the D42 background fails to extend life span, suggesting that the overlapping set of glutamate neurons has an important role in aging. Moreover, studies with 10-day-old D42-ETC RNAi showed increased sleep in light and dark cycles and decreased locomotor activity during dark cycles. Sleep and locomotor activity patterns were observed in glutamate-specific ETC RNAi in male flies aged 5 days and 30 days to determine the correlation between activity and aging. The results of this study contribute to the growing understanding of biological mechanisms influencing aging, specifically the role that Complexes I, IV, and V have on lifespan extension.

738S **Sex-specific effects of Superoxide Dismutase 1 knockdown on healthspan and lifespan** Denise P Horner[1], Nicole C Riddle[2] [1]Biology, University of Alabama at Birmingham, [2]University of Alabama at Birmingham

Superoxide dismutase 1, SOD1, is a mitochondrial enzyme that removes toxic superoxide anion radicals generated through electron transport leakage by metabolizing them into hydrogen peroxide and oxygen. SOD1 dysfunction is associated with several disease conditions such as premature aging, amyotrophic lateral sclerosis (ALS), and cancer. *Sod1* gene knockdown reduces lifespan, while *Sod1* gene overexpression increases lifespan in mice, flies, and nematodes. Using *Drosophila melanogaster*, we investigate the sex-specific effects of *Sod1* gene knockdown on healthspan and lifespan. We use RNAi and the UAS-Gal4 system to find that ubiquitous *Sod1* gene knockdown has a strong effect on lifespan and that this effect is sex-specific. Males lacking SOD1 show a shorter lifespan than control animals and females lacking SOD1. Females lacking SOD1 also show a reduced medium lifespan, but their maximum lifespan is similar to that of controls. Males lacking SOD1 show decreased activity levels, while in females lacking SOD1 activity levels were similar to those of control animals. These results suggest that males are more affected by the accumulated reactive oxygen species present due to lack of SOD1. Ongoing experiments explore the molecular mechanisms that control these sex-specific effects of *Sod1* gene knockdown.

739S **Effects of temperature and age on energetics of starvation-selected *Drosophila melanogaster*** Elena DeLaTorre[1], Katrina G Pinili[2], Allen G Gibbs[2] [1]University of Nevada, Las Vegas, [2]University of Nevada

We reared replicated populations of starvation-selected (SS) *D. melanogaster* at 18, 25 and 29 °C. SS flies eclosed with 2-3 times higher triglyceride content than their fed control (FC) counterparts. Lipid content did not differ among flies reared at different temperatures. Lipid content was assessed for 3 weeks in adults held at the same temperature as their rearing temperature. Adults maintained constant lipid content as they aged at 18 and 25 °C. At 29 °C, SS flies lost lipid stores as they aged, but FC flies did not. These results suggest that starvation-selected flies are more sensitive to high temperature stress.

740S **UDP-Glycosyltransferase UGT35B1 alters the response to nicotine in *Drosophila melanogaster*** Luke Pfannenstiel[1], Jeffrey Scott[2], Nicolas Buchon[2] [1]Entomology, Cornell University, [2]Cornell University

Multiple gene families including UDP-Glycosyltransferases (UGTs), cytochrome P450s, glutathione S-transferases, and ABC transporters have been shown to play a role in the metabolism and excretion of xenobiotic compounds in insects. We investigated the role of two of these families using RNAi knockdown in vivo in Drosophila. Specifically, we tested the role of ABC transporters in the susceptibility to acephate, imidacloprid and methomyl and the role of UGTs in response to nicotine. Our screen identified six ABC transporters which changed mortality to individual insecticides after RNAi knockdown and one UGT which increased mortality in response to nicotine. After further validating our candidate genes with additional RNAi knockdown and CRISPR knockout, we focused on *Ugt35B1* as knock down of this gene showed a strong increase in mortality and the role of this gene family is less studied. We followed up on this phenotype by investigating where Ugt35B1 is required for nicotine tolerance using tissue-specific RNAi. We also noticed that *Drosophila* larvae are more sensitive to nicotine exposure than adults, which may be due to the different expression patterns of *Ugt35B1* seen between larvae and adults. This difference in tolerance between life stages is investigated further using nicotine bioassays of larvae after *Ugt35B1* RNAi. Our results show that *Ugt35B1* plays a role in *Drosophila's* tolerance to nicotine and provide information about how *D. melanogaster* and potentially other insects deal with exposure to nicotine.

741S **Post-translational regulation of Pcyt1 to support phospholipid synthesis during the immune response in *Drosophila*.** Elizabeth Van Gorder[1], Michelle L. Bland[2] [1]Pharmacology, The University of Virginia, [2]Pharmacology, University of Virginia

Metabolic changes during infection influence the immune response. For example, secretion of immune effector proteins such as antibodies and antimicrobial peptides (AMPs) is accompanied by increased phospholipid synthesis that supports expansion of the endoplasmic reticulum (ER). Our lab has shown that both phospholipid synthesis and ER volume increase in response to innate immune signaling through the Toll pathway in the *Drosophila* larval fat body. Innate immune signaling induces expression of phospholipid synthesis enzymes, including choline-phosphate cytidylyltransferase A (Pcyt1), the rate-limiting enzyme in phosphatidylcholine synthesis. Loss of Pcyt1 in the *Drosophila* larval fat body delays bacterial clearance during infection. Increased phospholipid production during infection may support ER membrane expansion and therefore secretion of AMPs. We have shown that innate immune signaling via the Toll pathway in *Drosophila* specifically regulates phospholipid production by increasing both RNA and protein amounts of Pcyt1. However, it is not understood how innate immune signaling might regulate Pcyt1 in a post-translational manner. Pcyt1 is known to move from within the nucleoplasm onto the nuclear membrane whenever it is active, and my results show that Pcyt1 localizes more onto the nuclear membrane in response to Toll signaling. Pcyt1 is a multiphosphorylated enzyme, but the kinases and phosphatases involved in that phosphorylation are unknown. It is also unknown how that phosphorylation is regulated in response to Toll signaling. Our results show that phosphorylation of Pcyt1 Ser433 increases after prolonged Toll signaling. Using biochemical and genetic approaches, we are identifying in vivo regulators of Pcyt1 phosphorylation in response to immune activation as well as activators of cell growth that drive expansion of cellular and organelle membranes.

742S **Blue Light Exposure and Aging: Metabolic and Genetic Disruptions in *Drosophila melanogaster*** Jun Yang, YUJUAN SONG, Yanming Di, Jadwiga Giebultowicz, David A. Hendrix Oregon state university

With LEDs rapidly becoming the primary light source in the U.S. due to their cost-effectiveness and energy efficiency, their emission of substantial blue light (~450nm) raises significant public health concerns. Unlike other visible lighting, blue light exposure has been linked to retinal damage and potentially far-reaching systemic effects. Our research in *Drosophila melanogaster* reveals that blue light exposure not only accelerates aging—resulting in a marked 25% reduction in lifespan—but also induces widespread neurodegeneration, when compared to flies kept in darkness, suggesting extensive systemic impact.

Through metabolomic profiling using HPLC-MS, we discovered that blue light disrupts the homeostasis of key metabolites associated with energy metabolism, especially ATP synthesis. Further analysis of mitochondrial respiratory function showed that these metabolic disturbances correlate strongly with reduced Complex II activity, underscoring the critical role of mitochondria in mediating the effects of blue light exposure. Genomic studies identified broad changes in gene expression, including the downregulation of ribosomal proteins. Normally, ribosomal protein expression in early developmental stages of *Drosophila* shows a natural upward trend, but blue light was found to suppress this increase. This observation may suggest that blue light has a particularly adverse impact on developing organisms. Such downregulation, alongside alterations in metabolic genes, further highlights the potential systemic health risks associated with blue light exposure.

Additionally, we identified two significant DNA-binding motifs among upregulated DEGs. The first motif appears to bind from the *CoRest*, homologous to DOF1—a light-sensitive transcription factor in plants—hinting at a potential evolutionary connection in light-responsive pathways. The second motif is bound by *Xrp1*, suggesting a feedback loop that may amplify cellular stress responses to blue light.

With blue light exposure from screens and LEDs on the rise, we are extending our research to primary human fibroblast cells to assess whether similar regulatory mechanisms are conserved across species. Preliminary findings indicate overlapping gene networks, which may reveal biomarkers or therapeutic intervention points relevant to human health. This comprehensive study provides foundational insight into how chronic blue light exposure may influence cellular and genetic health, potentially contributing to age-related diseases.

743S **A neurodegenerative phenotype in survivors of Blm-deficient development in *Drosophila melanogaster*** Ava M Hasenoehrl, Brayden Graves, Tesla Presnell, Abigail Brown, Jayden Youngren, Eric Stoffregen Lewis-Clark State College

DNA damage caused by a lack of maternally loaded Blm protein during early embryonic development in *Drosophila melanogaster* results in significant embryonic lethality. It is unknown, however, how this DNA damage affects normal physiologic processes in the few surviving individuals. We investigated whether this developmental abnormality (Blm-deficiency induced DNA damage) causes neurologic dysfunction in adult survivors. We hypothesized that this DNA damage exposure during early development would cause reduced lifespan, loss of motor function, and disruption of normal sleep patterns and circadian rhythms. To test our hypotheses, we collected adult progeny from Blm^{-} mothers crossed to Blm^{+} fathers and from the reciprocal cross, Blm^{+} mothers crossed to Blm^{-} fathers. In both crosses, surviving progeny were heterozygous for *Blm*, but one set developed with maternally loaded Blm protein (from Blm^{+} mothers) and one without (from Blm^{-} mothers). We compared lifespan between these sets of progeny, used a climbing assay to measure motor function, and investigated sleep and circadian rhythms using a continuous activity monitor. Progeny that developed without Blm protein exhibit a significant reduction in lifespan, a significant decrease in climbing ability, a significant disruption in sleep condensation, and a significant change in circadian patterns compared to flies that developed with Blm protein. Since these phenotypes are commonly observed in old flies, we are assessing whether there are signs of advanced biological aging in the flies that develop without Blm.

744S **Defining the function of the GATOR2 complex in the regulation of TORC1 signaling** Chun-Yuan Ting, Richard Garcia, Natalie Rowland, Mary A. Lilly NICHD, NIH

The multiprotein Target of Rapamycin (TOR) Complex 1 (TORC1) is a serine/threonine kinase that stimulates anabolic metabolism and suppresses catabolism. Deregulation of TORC1 is implicated in various human pathologies, including cancer, epilepsy, and neurodegenerative disorders. The Gap Activity Towards Rags (GATOR) complex contains two subcomplexes: GATOR1, which inhibits TORC1 activity in response to nutrient starvation; and GATOR2, which counteracts GATOR1's function. Structural and biochemical studies have elucidated how GATOR1 regulates TORC1 activity by acting as a GTPase activating protein for Rag GTPase. However, while cryogenic electron microscopy has determined that the structure of the multi-protein GATOR2 complex is conserved from yeast to humans, how GATOR2 inhibits GATOR1 remains unclear. We will present our biochemical, genetic and computational analysis exploring how the GATOR2 complex inhibits GATOR1 to control growth, development and the response to stress.

745S **Metabolic dysfunction following Blm-deficient development in *Drosophila melanogaster*** Abigail P Brown, Ava Hasenoehrl, Eric Stoffregen, Connor Alexander, Jayden Youngren Lewis-Clark State College

Blm DNA helicase is essential for proper DNA replication during early development in Drosophila melanogaster. *Blm*$^{-}$ mothers, who do not provision their eggs with functional Blm protein, exhibit a maternal-effect lethality. Nearly all progeny from *Blm*$^{-}$ mothers die before larval hatching; however, a few survive to adulthood (<10% of embryos). These survivors provide a model to study the effects of DNA damage during early development on healthspan. We hypothesized that survivors of Blm-deficient development would display alterations in metabolic function. We first tested whether development without Blm protein affected body mass and determined that Blm-deficient development results in a statistically significant reduction in body mass. Our data also suggests that Blm-deficient development results in decreased energy storage in adult flies, with clear reductions in triglyceride levels and a possible reduction in glycogen storage as well. These data suggested that the DNA damage sustained by embryos lacking Blm during early cell cycles either affected metabolic processes related to energy storage or affected the feeding behavior of the flies. To test whether these differences in metabolism could be accounted for by food consumption differences, we performed capillary feeder (CAFE) assays and saw no difference in food consumption by flies that survived Blm-deficient development, suggesting instead that these flies exhibit defects in metabolic processes.

746S **Investigating the impacts of intestinal Snakeskin Knockdown on Protein Aggregation in Drosophila** Christina Richardson, Hunter Moore, Connor Auby, Anna Salazar Christopher Newport University

The connection between gut health and aging has gained significant attention recently, with evidence linking gut changes to known aging phenotypes, or hallmarks of aging, such as inflammation and neurodegeneration. Research in organisms like fruit flies, Drosophila melanogaster, has shown that intestinal barrier function, where stuff in the gut leaks out, plays a key role in overall health. As organisms age, the gut's structural integrity deteriorates, leading to a "leaky gut". In Drosophila, the cell junction protein Snakeskin, Ssk, is crucial for maintaining gut integrity by holding gut cells tightly together. With age, Ssk proteins decrease in amount, resulting in failure of the gut barrier. This results in aging-related phenotypes, such as dysbiosis (microbial imbalance), leaky gut, inflammation, diabetes, and mitochondrial dysfunction and protein aggregates in muscle tissue, ultimately resulting in death. Knocking down Ssk in young flies causes these aging phenotypes in young animals and severely decreases the lifespan of the flies. Interestingly, these phenotypes can be completely reversed by restoring Ssk, allowing the intestinal barrier to become intact. Overexpressing Ssk improves barrier function, reduces aging symptoms, and extends lifespan. Current experiments investigate how Ssk manipulation in the gut affects protein aggregates and mitochondrial health in the muscles, as well as climbing behavior, which is a measure of muscle function in fruit flies. The ultimate objective of this research is to be able to better understand how changes in the gut can impact tissue outside of the gut, like muscles, and lead to therapies that delay the aging process.

747S **Identifying and characterizing Curcumin as a natural compound that promotes longevity and healthspan in *Drosophila melanogaster*** Naomi Z Serrano Colón, Imilce Rodríguez Fernandez Biology, University of Puerto Rico, Río Piedras campus

Aging is a natural, progressive decline in tissue, organ, and organismal function. It is the greatest risk factor for many chronic diseases. Since the world population is aging, this has become an increasingly urgent public health challenge requiring innovative strategies for the prevention and treatment of age-related diseases.

We aim to identify new interventions that can promote the healthspan (the disease-free period of life) and/or lifespan (maximum longevity) extension of animals by exploring the world of natural products as a source of novel compounds. To identify new compounds, we have developed an affordable, high-throughput screening platform that will allow us to quickly identify compounds that lead to Drosophila melanogaster healthspan by using the 'smurf assay' as a read-out. The 'smurf assay' quickly measures gut barrier dysfunction by feeding flies food mixed with blue dye No.1; if the gut is leaky, the flies turn blue, indicating imminent death.

We are validating this platform by testing published beneficial and detrimental compounds. Of particular interest is curcumin, a polyphenolic substance found in Turmeric-root that has been reported to lead to lifespan extension in adult Drosophila via an unknown mechanism. To understand the effects of Curcumin in barrier function using the Smurf assay, we used wild-type Canton-S flies and fed them Curcumin (0 µM, 62.5 µM, 125 µM and 500 µM in 0.5% DMSO) for 3 days and then exposed the flies to 25 mM Paraquat to induce oxidative stress to mimic the aging condition. We found that 500 µM curcumin can rescue young wild-type flies from oxidative stress damage and barrier dysfunction. Similarly, using a model of accelerated aging known as SODn1 heterozygous mutants, we found that 500 µM curcumin can protect from oxidative stress-related barrier dysfunction.

Current work is focused on characterizing the molecular mechanism behind curcumin's longevity and health-span effects. To understand these effects, we used wild-type old Canton-S flies (55d) and fed them Curcumin at 0 µM, 62.5 µM, 125 µM and 500 µM in 0.074% DMSO for 3 weeks. Each week flies in treatment were used to perform the following assays: Smurf, climbing, and Colony Forming Unit (CFU). Curcumin concentration at 125 µM seems to reduce leaky gut, alter the gut microbiome, and improve climbing ability when adult flies are treated during old age. Our findings shed light on the mechanistic understanding of the beneficial properties of Curcumin.

748S **Comparing the effects of continuous vs. intermittent hypoxia provides genetics insights in *Drosophila*** Miled A. Maisonet Nieves[1], Katherine Warren[2], Arianna Smith[3], Laura K Reed[1] [1]Biological Sciences, University of Alabama Tuscaloosa, [2]University of Alabama Tuscaloosa, [3]Mechanical Engineering, University of Alabama Tuscaloosa

Hypoxia is a condition characterized by oxygen levels that are too low to maintain adequate homeostasis at the tissue level. Even though hypoxia is a common disorder, there are multiple causes, such as underlying illnesses like chronic obstructive pulmonary disease, pneumonia, fibrosis, and anemia that negatively impacts blood flow and breathing. Oxygen is an important molecule for cellular function, as cells use oxygen to produce energy as ATP. The association between low oxygen levels and the progression of severe human health conditions is evident, particularly within the tumor microenvironment, where hypoxia plays a role in initiating tumor formation, promoting angiogenesis, developing resistance to drugs, and facilitating the spread of cancer. It has been shown that some conditions like hypoxia and metabolic disorders suppress the expression of genes involved in metabolic processes in the mitochondrion. However, there is little understanding about the molecular pathway and changes during hypoxic stress and how it affects metabolism. The development of an experiment using fruit flies in hypoxic conditions could provide insights on how low oxygen levels influence the metabolism of people with acute hypoxia. For this study, we induced hypoxia using a custom chamber in different wild-type Drosophila Genetic Reference Panel (DGRP) lines to characterize and analyze genetic variation across phenotypes. We also studied the activity and mortality rates between flies treated with continuous hypoxia versus intermittent hypoxia. We hypothesize that there will be genetic variation across different phenotypes influenced by hypoxia. Further, we propose that inducing continuous hypoxia for three consecutive days will result in higher mortality rates in *D. melanogaster* compared to intermittent hypoxia (three days of hypoxia interspersed with reoxygenation periods), which will reduce stress and thereby increase the longevity of the flies. Studying the impacts of low oxygen conditions is important because such analyses will provide a better comprehension of how different environmental states affect health conditions.

749S **Characterizing the molecular impact of social defeat on the male *Drosophila* midgut: A transcriptomic and proteomic approach** Audrey E Parkey[1], Deanna Cuello[1], Solange Holman[1], Erin Salda-petree[2], Sarah Certel[2] [1]Neuroscience, University of Montana, [2]University of Montana

Aggressive encounters can have lasting effects on individuals, including in humans, which include anxiety- and depression-related behaviors, immune system dysregulation, and gut dysfunction. In all systems, physical stress as well as bacterial metabolites can lead to various alterations in normal gastrointestinal (GI) function and signaling. An often overlooked, underlying aspect of gut-brain signaling and gastrointestinal physiology, is sex, i.e. whether a cell is intrinsically male or female. Sexually dimorphic changes in gene expression impact growth, metabolism, and neurotransmitter signaling. Our overarching goal is to understand how social stress alters gene expression, gut-brain signaling and ultimately behavior in both males and females. To start to accomplish this goal, we are identifying transcriptomic and proteomic changes in males that have experienced the stress of losing aggressive encounters.

For our RNA-seq analysis, we paired socially naïve wild-type Canton-S (CS) males for three sequential 20-minute fights with a 10-minute separation between fights. Video analysis of the fights identified a "loser" male. The midguts of losers and controls were dissected 24 hours following the third fight. Selected gene changes include a downregulation of CG13646, a member of the solute carrier (SLC) protein family, and *polyph*, an L-amino acid sensor essential for immune defense. These findings indicate stress-induced transcript shifts in GI function; however, it is still unknown if these changes extend to the protein level. To address this question, we are using mass spectrometry (MS). Specifically, socially naïve wild-type Canton-S (CS) males will be paired with "bully" males. Midguts of losers, winners and controls will be dissected two hours post-fight. Proteins will be extracted and undergo MS-based proteomic analysis. A combination of univariate and multivariate statistical analyses will be employed to identify candidate protein biomarkers of social stress and elucidate protein expression patterns unique to the "loser phenotype." Results from this study will provide foundational knowledge of the molecular and cellular codes underlying internal state changes that occur as a result of social stress and defeat.

750S **Strengthening Gut Barrier Integrity through Overexpressing Snakeskin** Curtis Patton, Concepcion Ibarra, owen Henke, Anna Salazar Christopher Newport University

Intestinal barrier function is associated with changes in health and aging, with gut integrity linked to lifespan in numerous organisms, including humans. Recent studies have utilized Drosophila melanogaster as a model organism to study the effects of intestinal barrier permeability on several markers of aging. Snakeskin (Ssk) is expressed between adjacent gut epithelial cells and is a septate-specific occluding junction protein that ensures the integrity of the gut by maintaining the barrier between the intestines and the rest of the body. These junctional proteins between intestinal epithelial cells play a major role in controlling the ability of bacteria, and other inflammation-inducing molecules, to pass from inside the gut to outside the gut, thereby limiting microbial dysbiosis and preventing the 'leaky gut' phenotype. Previous work has shown that overexpressing Ssk leads to a decrease in intestinal barrier dysfunction, a decrease in microbial dysbiosis, and an increase in the lifespan of the fly. This research utilizes the GeneSwitch Gal4/UAS system in order to overexpress Ssk in *Drosophila*, in a tissue-specific manner, in order to examine its effects on markers of aging, such as protein aggregation and mitochondrial dysfunction. The goal is to understand whether increasing intestinal barrier function can be a possible treatment for diseases associated with aging in humans.

751S **Investigating the role of Sirt6 in protein translation and proteostasis during aging.** Roja Sharma[1], Samarth Khanna[1], Arjun Khanna[2], Brianna Breazu[2], Samira Xhaferi[2], Jackson Taylor[1] [1]Center for Gene Regulation in Health and Disease, Department of Biological, Geological and Environmental Sciences, Cleveland State University, [2]Cleveland State University

Investigating the role of Sirt6 in protein translation and proteostasis during aging.

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Sirt6 is a member of the sirtuin family of NAD+-dependent deacetylase proteins and regulates a variety of cellular functions including glucose and lipid metabolism, DNA repair, and gene expression. Multiple studies have shown that Sirt6 regulates aging; for example, in mice and flies, reducing Sirt6 levels shortens lifespan, while overexpressing (OE) Sirt6 extends lifespan, indicating a conserved role for Sirt6 as a pro-longevity gene. Although Sirt6 has been in the spotlight of many age-related studies, the complete mechanisms by which Sirt6 regulates lifespan are not yet fully understood. Previous studies have reported that Sirt6 OE reduces protein synthesis while Sirt6 knockout increases protein synthesis. Interventions which decrease protein synthesis have been shown to correlate with slowed aging, possibly through reduction in protein aggregate accumulation. Of the seven sirtuin genes present in mammals, five are present in Drosophila melanogaster, including Sirt6, though few studies exist on the function of Sirt6 in flies. Here, we used Sirt6 deletion (Sirt6 -/-) and overexpression flies to investigate the tissue specific role of Sirt6 in protein synthesis and proteostasis during aging. We found that Sirt6 -/- flies have shortened life span, higher body weight and increased H3K9 acetylation. Interestingly, we also saw that Sirt6 -/- flies have significantly reduced survival during heat stress, while Sirt6 OE flies have improved survival, versus controls. Sirt6 -/- flies also have altered levels of global protein synthesis, as determined by SUnSET assay. RNA-seq results show that Sirt6-/- flies have increased expression of ribosome biogenesis genes. In addition, Sirt6 -/- flies have reduced levels of Thor (4E-BP) mRNA, a repressor of translation initiation, while Sirt6 OE flies have increased levels of Thor mRNA. Together these results suggest multiple mechanisms by which Sirt6 may regulate protein synthesis. We also see an increase in protein aggregates in Sirt6-/- flies, indicated by increased levels of ref(2)P (fly homolog of p62) and polyubiquitin. Collectively, our findings provide additional evidence that Sirt6 is a key regulator of protein synthesis and also suggest a novel role for Sirt6 in maintaining proteostasis in Drosophila. These findings provide a new potential mechanism by which Sirt6 may extend lifespan.

752S **Translator Functional analysis of the cardiogenic roles of *spalt major* and *spalt-related*, *Drosophila* orthologs of human zinc finger transcription factor-encoding genes associated with congenital heart defects** Mofazzal Karim Sabbir[1,1,2], Karim Zaher[1], M. Rezaul Hasan[1,2], Rajnandani Katariya[1,1,2], Kuncha Shashidhar[1], Shaad M. Ahmad[1,2,3] [1]Biology, Indiana State University, [2]The Center for Genomic Advocacy, [3]Rich and Robin Porter Cancer Research Center

Mutations in the human zinc finger transcription factor-encoding genes *SALL1* and *SALL4* lead to Townes-Brocks Syndrome and Duane-radial ray Syndrome (Okihiro Syndrome) respectively, both of which exhibit congenital heart defects (CHDs). Given the conservation of genetic pathways in heart development between mammals and fruit flies, we have begun to functionally analyze the cardiogenic roles of *spalt major* (*salm*) and *spalt-related* (*salr*), the *Drosophila* orthologs of these mammalian *SALL* genes, in an attempt to shed light on these CHDs. The proximity and potential redundancy between *salm* and *salr*, which are paralogous genes separated by a small intergenic region, complicate the analysis of their individual functions. We therefore employed CRISPR/Cas9 technology to generate null mutations in *salm* and *salr* and are using these alleles, along with the *Df(2L)Exel6029* deficiency which deletes both these *spalt* genes, to examine the roles of the *spalt* genes both individually and in concert. In wild-type *Drosophila* embryos, the heart is a tubular structure closed at the posterior end, composed of two rows of bilaterally symmetrical myocardial cells, with contralateral hemisegments of these cells from the left and right sides of the embryo pairing and aligning perfectly along the dorsal midline. In contrast, embryos lacking *spalt* functions reveal multiple cardiac defects, including misalignment of contralateral myocardial hemisegments, abnormal curvature of the heart tube, uncharacteristic clustering of Tin-expressing myocardial cells at abdominal segment seven, and incomplete closure of the posterior heart tube. We intend to utilize real-time live imaging of the developing heart to assess the precise timing, location, and cause of these defects. By understanding the roles of *salm* and *salr* in *Drosophila* heart development, we aim to provide critical insights into the conserved genetic mechanisms underlying the CHDs in Townes-Brocks and the Duane-radial ray Syndromes.

753S **The transcription factor Jim regulates lifespan and lipid metabolism in *Drosophila melanogaster*** Jackson Taylor[1], Roja Sharma[1], Samira Xhaferi[1], Neelanjana Roy[1], Prema Singaravel[1], Arjun Khanna[1], Samarth Khanna[1], Evan Mizerak[2], Dowon Kim[2], Julianna Liu[2], Hanna Wang[2], Stephen Helfand[2] [1]Center for Gene Regulation in Health and Disease, Department of Biological, Geological and Environmental Sciences, Cleveland State University, [2]Department of Molecular Biology, Cell Biology, and Biochemistry, Brown University

Aging is characterized by complex remodeling of the epigenome, including loss of repressive heterochromatin that may subsequently lead to aberrant gene expression and de-repression of retrotransposons (RTE). However, how specific perturbations to heterochromatin affect the aging process are not fully understood. Using the model system *Drosophila melanogaster*, we identified the zinc-finger transcription factor *Jim* in a genetic screen for genes that are necessary for RTE silencing. We found that reducing *Jim* expression enhances RTE mobilization in an RTE reporter line and leads to strong transcriptional up-regulation of the RTE *copia*. *Jim* overexpression increases silencing in a heterochromatin reporter line and increases H3K9me2 levels, a histone modification associated with heterochromatin. Both Jim overexpression and Jim knockdown by RNAi dramatically shorten lifespan in both sexes. Interestingly, while Jim knockdown strongly reduces survival time under starvation conditions, Jim OE significantly increases starvation resistance in male flies, suggesting an additional role in lipid metabolism. Oenocyte (analogous to liver in flies)-specific *Jim* overexpression also shortens lifespan and produces major deficits in desiccation resistance and cuticle waterproofing. Transcriptomic profiling of fat-body tissue revealed that *Jim* regulates the expression of several thousand target genes, which were highly enriched for function in lipid and carbohydrate metabolism pathways. We conclude that *Jim* is a major regulator of heterochromatic silencing, metabolism, and lifespan.

754S **The Fire Gene Complex is Mediating Iron Absorption in *Drosophila melanogaster*** Mayen Kalu, Sattar Soltani, Kirst King-Jones Biological Sciences, University of Alberta

Iron is a paradoxical element—essential for life, yet challenging to absorb. Critical for oxygen transport, DNA synthesis, and energy production, its limited bioavailability and low solubility requires specialized mechanisms for its absorption, transport and storage. Through a multi-generational iron starvation and re-feeding approach coupled with RNA-Seq analysis, we identified two ferric reductases and a cytochrome b5 protein (termed the fire gene complex – *fire, fire-like* and *firewood*), suggesting a role in dietary iron absorption. These genes are strongly downregulated when iron-starved larvae are switched to an iron-rich diet. *Fire* and *fire-like* encode CYB561 enzymes and are predicted to reduce dietary ferric iron (Fe^{3+}) to the more absorbable ferrous form (Fe^{2+}), which is then taken up by the divalent metal transporter Malvolio. We hypothesize that Firewood serves as an electron donor for Fire and Fire-like. The fire gene complex appears to be co-regulated, and all three genes have human orthologs, suggesting an evolutionary conserved mechanism in the gut.

Functional assays confirmed that ferric reduction in the gut was abolished in *fire* and *fire-like* double null mutants, with no apparent compensation by other CYB561 members, indicating that Fire and Fire-like are critical for iron absorption. *Fire* and *fire-like* double null mutants display reduced survival on diets supplemented with iron chelators or antioxidants (to neutralize reducing compounds in the diet) since they are unable to reduce ferric to ferrous iron. We are currently investigating whether loss-of-fire/fire-like function can be rescued with transgenic expression of human CYB561 homologs. We are also developing individual CRISPR/Cas9 knockouts of *fire*, *fire-like*, and other genes acting in iron absorption, to dissect the specific mechanism by which dietary iron enters the fly gut.

While this study focuses on iron absorption in insects, it may also broaden our understanding of human iron uptake into enterocytes. In humans, the equivalent gene, DCYTB, is likely not the only CYB561 enzyme involved in iron absorption, suggesting that Fire/Fire-like orthologs may also contribute to dietary iron uptake.

755S **Transcriptomic effects and biological consequences of reducing DNA Polymerase α during stress and aging in *Drosophila*** Logan Wallace Shepard[1], Yanyan Qi[2,3], Hongjie Li[2,3], Xin Chen[1,4] [1]Department of Biology, Johns Hopkins University, [2]Department of Molecular and Human Genetics, Baylor College of Medicine, [3]Huffington Center on Aging, Baylor College of Medicine, [4]Howard Hughes Medical Institute

DNA replication is a universally essential process that must be tightly regulated to ensure genetic information is faithfully copied. Yet, it is not well understood how DNA replication contributes to cell fate decisions in multicellular organisms, and how this regulation may change in response to different stress conditions. Previously, we found that *Drosophila* with reduced expression of the DNA replication component DNA Polymerase α (Polα) are more fertile during aging without any compromise to lifespan (Ranjan R et al. 2024 *bioRxiv*). Furthermore, upon introducing stressors such as genetic ablation of the male early germline or bacterial infection, *polα* heterozygotes exhibit enhanced regeneration of the germline and prolonged survival, respectively (Ranjan R et al. 2024 *bioRxiv*). Despite these intriguing observations, the underlying mechanisms remain unclear. Using single-nucleus RNA sequencing (snRNA-seq), I will generate and compare the whole-body transcriptomic atlas of both control and *polα*$^{+/-}$ flies during aging. In the future, I will also perform similar experiments to compare these two genotyped flies under stressed and unstressed conditions throughout aging. For these experiments, I am using a workflow similar to that published in the Aging Fly Cell Atlas (Lu TC et al. 2023 *Science*). Through these genomic studies, I will identify candidates and validate them through orthogonal approaches, such as RNA FISH and immunostaining. I will further manipulate potential target genes by loss-of-function and gain-of-function assays to understand functional connections of DNA replication, cell fate decisions, and organismal health under physiological and pathological conditions. This work will help our understanding of the roles DNA replication plays in cell fate determination and cellular plasticity, under stress and aging, across distinct tissues and cell types in *Drosophila* at the whole-body level.

756S **Effects of smoking and obesity on respiratory and organismal health across generations** Ann-Cathrin Hofacker[1], Susanne Krauss-Etschmann[2], Thomas Roeder[1] [1]Molecular Physiology, Christian-Albrechts-University Kiel, Germany, [2]PRA Chronic Lung diseases, Research Center Borstel

The main characteristics of the so-called Western lifestyle, which is widespread in industrialized societies, are a combination of a sedentary lifestyle and a high-calorie diet that is characterized by high levels of sugar and fat. This unfavorable lifestyle in early and later life impairs lung development and significantly increases the risk of cardiovascular and pulmonary diseases. Moreover, cigarette smoke substantially aggravates these pathological developments. We used the fruit fly *Drosophila* as a model to study the effects of high-calorie diets in combination with cigarette smoking and physical activity. In the intragenerational setting, a high-calorie diet and smoking significantly shorten the lifespan of flies. The combination of smoking and a high-calorie diet aggravated the lifespan shortening. Impaired body composition and fitness parameters induced by a high-calorie diet were usually aggravated by cigarette smoking. Moreover, a high-calorie diet decreased physical activity dramatically, and the combination of a high-calorie diet and cigarette smoking increased the susceptibility to airborne stressors. Interestingly, both interventions increased stem cell proliferation and reduced gut health. The analysis of the effects on the airway structure and the airway progenitor cells in the intra- and transgenerational setting is currently underway.

757S **Importance of hormonal regulation in the intestinal tract of flies** Stina Madeline Bettendorf, Thomas Roeder Molecular Physiology, Christian-Albrechts-University Kiel, Germany

Hormonal regulation via second messenger signaling plays a crucial role in regulation of cellular functions. Until now, there is a lack of knowledge towards the hormonal signaling in enterocytes (EC). The aim of this project is to better understand the complex interplay between the microbiota and the intestine mediated by hormons, focussing on the hormonal regulation of the intestinal physiology. In turn, we manipulated the most important second messengers Ca^{2+} and cAMP and the Dopamin 1 Receptor 1 and the Dopamin 1 Receptor 2 using optogenetic tools in the enterocytes of the intestine and monitoring the effects on the phenotype of the fly and its intestinal microbes.

758T **Defining Activities of the KDM5 C-terminus Essential to Development and Viability** Melissa Castiglione[1], Julie Secombe[2], Andreas Bergmann[3], Hans Martin-Herz[4] [1]Genetics, Albert Einstein College of Medicine, [2]Genetics, Neuroscience, Albert Einstein College of Medicine, [3]UMass Chan, [4]Cell and Molecular Biology, St. Jude Children's Hospital

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. KDM5 family proteins are named for their demethylase activity, which targets the active chromatin mark H3K4me3. However, KDM5 proteins can also regulate transcription through non-enzymatic mechanisms. While KDM5 is essential for development, its demethylase activity is not required, as is demonstrated by the viability of demethylase-dead adult flies. In this work, we will examine essential functions of the C-terminus of KDM5 via a novel truncation allele, *Kdm5*^{Q19}, which modestly alters the H3K4me3 landscape, but does not abrogate demethylase function. *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 demethylase dead and other mutants generated in our lab, suggesting that the region disrupted by this truncation plays an essential as-yet-unknown role in normal KDM5 function. To further dissect the molecular activities of this region of KDM5, we have generated additional alleles of *Kdm5* to refine the critical region(s) of the protein and assess viability and changes to transcription.

In this work, we aim to (1) define essential regions within KDM5 required for viability, and to characterize their roles in development (2) define the essential molecular functions imparted by the C-terminus of KDM5. Together, these studies will refine the critical region(s) of the C-terminus of KDM5 and define their role in regulating transcription.

759T **Nutrient-dependent regulation of the Drosophila melanogaster Estrogen-Related Receptor (ERR)** Sophie A Fleck, Hongede Li, Maria Sterrett, Jason Tennessen Department of Biology, Indiana University Bloomington

Animal development is acutely sensitive to environmental conditions including dietary nutrients, temperature stress, and xenobiotic compounds. One of the key sensors involved in coordinating environmental cues with developmental events are the highly conserved family of nuclear receptor transcription factors. In this regard, the Estrogen-Related Receptor (ERR) family of orphan nuclear receptors play a key role in coordinating cellular metabolism with developmental gene expression programs. We previously demonstrated that the *Drosophila melanogaster* ortholog of ERR activates a metabolic program known as aerobic glycolysis prior to the onset of larval development, thus establishing a metabolic state that supports rapid juvenile growth. During this embryonic switch, which occurs approximately 12 hours before hatching, accumulation and activation of ERR protein directly activates transcription of genes encoding enzymes within glycolysis and the pentose phosphate pathway (PPP). Intriguingly, we've found that expression of ERR protein earlier in embryonic development does not drive precocious activation of aerobic glycolysis. However, premature expression of a constitutively active ERR-VP16 fusion protein is capable of inappropriately activating target gene expression. Together, these findings indicate that ERR activity is controlled at a post-translational level via an unknown mechanism. Consistent with these embryonic studies, we've also demonstrated that ERR activity during larval development tightly correlates with nutrient availability, as ERR become inactive when *Drosophila* larvae are fed a nutrient poor diet. Further analysis by western blot revealed an approximate 40 kDa shift in the weight of ERR when larvae are starved. These findings indicate that ERR may be regulated through inhibitory post-translational modification(s) (PTMs) when environmental conditions are inadequate for *Drosophila* development. Considering that ERR family members are orphan receptors that are highly conserved across evolutionary time, our findings are important because they hint at an unknown mechanism that controls post-translational ERR activity. Finally, our studies indicate that ERR coordinates developmental metabolism with nutrient availability.

760T **Mammalian OVO-Like Transcription Factors Rescue Drosophila OVO at Both the Phenotypic and Transcriptional Levels in the Female Germline** Leif Benner[1], Lorielle Raab[1,2], Charli L Wingfield[1,2], Savannah Muron[2], Jillian G Gomez[2], Brian Oliver[2], Leah F Rosin[1] [1]NICHD, National Institutes of Health, [2]NIDDK, National Institutes of Health

The OVO and OVO-Like (OVOL) genes are a class of zinc-finger transcription factors conserved across metazoan. Drosophila OVO is a transcriptional activator expressed and required in the female germline, and the somatically expressed OVO, also known as *shavenbaby* (SVB), is required embryonically for denticle belt formation. Mice and humans contain three OVO-Like genes (OVOL1, OVOL2, OVOL3) that have been described as transcriptional repressors and have been shown to be required for primordial germ cell specification and hair formation in mice, phenotypically similar to the roles of Drosophila OVO and SVB. We conducted a conservation analysis of Drosophila and mammalian OVO/OVOLs at the protein level and found that OVO/OVOLs show a high degree of conservation in the four annotated zinc-fingers. However, mammalian OVOLs are on average 80 kDa smaller than Drosophila OVO and the repressive Snail/Snag domain N-terminally located in mammalian OVOLs is not conserved in Drosophila. The conserved zinc-fingers of OVO/OVOL have an almost identical DNA binding motif and Drosophila OVO binds to the same DNA binding motifs of mammalian OVOLs *in vivo*. We have previously shown that Drosophila OVO binds to and positively regulates a number of genes in the female germline that are required for oocyte differentiation and embryonic development. Hypomoprhic *ovo* alleles have a decreased expression of these required oocyte/maternally deposited genes which results in an arrested egg chamber phenotype and sterility. We expressed all three mouse (mOVOL) and human (hOVOL) OVOLs in the Drosophila female germline in an *ovo* hypomorphic genotype and found that mOVOL/hOVOLs were able to rescue the arrested oogenesis phenotype. Female Drosophila expressing OVOL1 and OVOL2 were able to lay eggs, however, the laid eggs did not hatch, indicating that this high degree of rescue was not total. RNA-seq profiling of Drosophila ovaries expressing mOVOL1/2/3 indicated that mOVOL1/2 were able to activate the expression of a vast majority of the OVO target genes in the female germline. Notably, this included genes involved in anterior/posterior/germ plasm specification (*bcd, exu, swa, osk, nos, pgc, gcl*) and egg activation (*png, plu, gnu, wisp, C(3)g, mtrm*). However, mOVOL3 activated the expression of a smaller subset of OVO target genes, consistent with the observed phenotypes. This indicates that in the context of the Drosophila female germline, mammalian OVOLs can act as transcriptional activators and retain an overall conserved role in regulating gene expression in the germline.

761T **Transcriptional co-repressor Atrophin regulates Hippo pathway target genes** Deimante D Mikalauskaite, Cordelia Rauskolb, Tom Lehan, Srividya Venkatramanan, Kenneth D Irvine, Deimante Mikalauskaite Department of Molecular Biology and Biochemistry, Waksman Institute, Rutgers University

The Hippo signaling pathway controls expression of growth-promoting target genes through its downstream effector, the transcriptional co-activator Yorkie (Yki). Experimental evidence suggests the existence of another transcriptional regulator of Hippo pathway target genes. Previously it was shown that the transcriptional co-repressor Atrophin (Atro) could control expression of Yki target gene *four-jointed*, we therefore hypothesized that Atrophin regulates expression of Yki target genes.

Using gene knock down and overexpression approaches we investigated whether Atro contributes to the Hippo signaling in the wing imaginal disc, and what molecular mechanisms underlie this regulation. We found that Atro affects expression of multiple Hippo pathway target genes. Interestingly, our results show that Atro represses transcription of Yki target genes in the distal wing while activating them in the proximal wing. Because Atro's activity shows spatial differences in Yki target gene expression, we investigated Atro's effect on Yki. Atro knock down resulted in decreased Yki nuclear levels, while overexpression had an opposite effect, suggesting that Atro controls Yki nuclear localization. This result implies that Atro also controls Hippo pathway activity. We identified that this regulation occurs through Atro's effect on the components of the Fat-Dachsous (Ft-Ds) pathway, which is an upstream regulator of the Hippo signaling. We observed that Atro interacts genetically with the transcriptional co-activator Vestigial (Vg) to control expression levels of *dachsous* (*ds*) gene, which encodes a component in the Ft-Ds pathway. Ds is an atypical cadherin, which interacts with another atypical cadherin Fat in a heterophilic manner to regulate Hippo pathway activity. In addition, Atro and Vg form a physical complex, which we hypothesize plays a repressive role in *ds* transcriptional regulation.

Current and future work will consist of identifying *ds* regulatory sequences through which Atro-Vg complex might suppress its expression. These studies will help us to better understand how Atro exerts its regulatory function on the components of the Ft-Ds network and explain the downstream effect on Yki target gene expression.

762T **Hippo Signaling role in *Drosophila melanogaster* cuticle pigmentation and dopamine metabolism** Shelley B Gibson[1,2], Samantha L Deal[2,3], Shinya Yamamoto[2,4,5] [1]Molecular and Human Genetics, Baylor College of Medicine, [2]Jan and Dan Duncan Neurological Research Institute, Texas Children's Hospital, [3]Program in Developmental Biology, Baylor College of Medicine, [4]Baylor College of Medicine, [5]Department of Neuroscience, Baylor College of Medicine

Highly conserved processes, such as the dopamine synthesis and the Hippo signaling pathways, can be studied in *Drosophila melanogaster* by observing pigmentation and growth phenotypes. Melanization of the fruit fly cuticle requires dopamine as a precursor of melanin and we found that inhibiting all four key kinases (*hippo*, *salvador*, *mats*, and *warts*) of the Hippo signaling pathway not only leads to the expected overgrowth phenotypes, but also an increase in pigmentation of the cuticle. This effect can be suppressed by the inhibition of both *yorkie* and *scalloped*, suggesting that canonical Hippo signaling is regulating both phenotypes. Expressing overactivated Yorkie also recapitulates the overgrowth and increase in pigmentation, further solidifying Hippo signaling's role in these processes. Additionally, *warts* knockdown cuticle phenotypes are completely rescued by overexpression of human homologs, *LATS1* and *LATS2*, supporting that molecular functions of these genes are conserved. Hippo signaling regulation of melanin levels may be through regulation of dopamine levels as HPLC measurement of dopamine shows increased levels in the fly head with *warts* knockdown in dopaminergic cells. As overexpressing the key enzymes of the dopamine synthesis pathway [Tyrosine hydroxylase (TH) and Dopa Decarboxylase (Ddc)] does not lead to an increase in pigmentation, we performed single-cell RNA sequencing in order to identify other potential targets of the Hippo signaling pathway in dopamine and melanin synthesis regulation. We are further performing a suppression screen using the *warts* knockdown cuticle pigmentation and growth phenotypes to identify previously unknown genes in each of these processes that are regulated by the Hippo signaling pathway. Both the dysregulation of dopamine as well as the Hippo signaling pathway are implicated in and cause a wide range of diseases or disorders that greatly impact human health. The fly cuticle is a useful model to identify potential causative variants or genes in the regulation of both of these processes with the ultimate goal of developing useful therapeutics.

763T **Investigating factors and interactions that coordinate histone gene expression** Casey A Schmidt[1], Shilpi Verghese[2], Leila Rieder[2] [1]Biology, Lafayette College, [2]Emory University

Expression of the replication-dependent histone genes is tightly controlled. Transcription is restricted to S-phase of the cell cycle, and histone mRNAs are quickly degraded upon the completion of DNA synthesis. In addition, histone transcripts are not polyadenylated. Instead, they terminate in a conserved 3' stem loop, and are the only known eukaryotic mRNAs to not have a polyA tail. To meet these unique transcriptional and processing requirements, a collection of factors known as the histone locus body (HLB) forms at the replication-dependent histone gene locus and coordinates histone mRNA production. Although many HLB members have been identified and characterized, including the core scaffolding protein Mxc, the full suite of factors remains unknown. Furthermore, it is unclear how these factors collaborate to coordinate histone gene expression. To expand the list of known HLB members, we utilized an *in vivo* proximity labeling approach. We targeted the promiscuous biotin ligase TurboID to the HLB and identified biotinylated proteins by mass spectrometry. Our initial analyses revealed several intriguing candidates for follow-up analysis. Our current efforts involve experimentally confirming proteomics data, cross-referencing with publicly available ChIP-seq data, and prioritizing candidates for further experiments.This screen is the first to address HLB composition in the context of development, and will allow us to further elucidate the unique mechanisms of histone gene expression.

764T **Natural variation in regulation of sexually dimorphic gene expression by the insulin signaling pathway in *Drosophila melanogaster*** Nafiul Huda, MD Mursalin M Khan, Rita M Graze Biological Sciences, Auburn University

Insulin signaling plays a role in regulating sex differences in gene expression and contributes to sexual dimorphism in complex traits; however, sex differences in the impact of regulatory variants in this pathway and downstream targets are less well understood. In this study, we examine the impact of insulin signaling on expression in diverse genetic backgrounds in each sex. We identify variation in the relationship between insulin signaling and sex differences in gene expression, as well as cross-sex genetic correlation in expression of downstream targets of the pathway. Comparing between control and perturbation conditions can address how the insulin signaling pathway may mediate sexual conflict in different environmental conditions.

765T **Identification of novel target genes and binding sites of NF-kB homologs Dif and dorsal in *Drosophila* larval fat bodies** Miyuki Suzawa, Michelle L Bland Pharmacology, University of Virginia

Gram-positive bacterial or fungal infections activate the Toll signaling pathway through two NF-κB transcription factors, Dif and dorsal, driving the synthesis of antimicrobial peptides that mediate the humoral immune response in *Drosophila*. We previously reported that Toll signaling in the larval stage suppresses whole-animal growth, reducing adult wing size by 12-15% in animals with constitutively active Toll^{10b} in the fat body. To identify molecules controlling peripheral growth in response to Toll signaling in fat body, we performed RNA sequencing to determine the genes regulated by acute expression of Toll^{10b} in the fat body. Through this approach, we identified *Drosophila insulin-like peptide 6 (Dilp6)* as a selective target of Toll signaling in the fat body. *Dilp6* expression was reduced by both acutely and chronically activated Toll signaling in fat body. Similarly, *Dilp6* mRNA levels were decreased by the expression of either Dif or dorsal in fat body. Interestingly, both Dif and dorsal are required to impair whole-animal growth in response to fat body Toll signaling, but only Dif functions in the cell-autonomous inhibition of insulin signaling. To understand how Dif or dorsal control target genes, we performed RNA-seq following acute expression of Dif or dorsal in the larval fat body. Interestingly, Dif and dorsal have very different effects on the fat body transcriptome, despite sharing nearly identical NF-κB response elements. Additionally, we found that Dif positively regulates phospholipid synthesis, and negatively controls genes involved in one-carbon metabolism glycolytic and the citric acid cycle; however, these genes were not regulated by dorsal in the fat body. We are addressing the following questions: 1) Which genes are directly regulated by Dif and/or dorsal in *Drosophila* larvae? 2) What are the binding sites that positively/negatively regulate genes by Dif and/or dorsal? 3) Is Dilp6 directly regulated by Dif? To answer these questions, we are working to identify genes directly regulated by Dif and dorsal in *Drosophila* larval fat body tissue using CUT&RUN technique. Our work has identified non-canonical targets of the Toll signaling pathway and highlighted the unique roles of Dif and dorsal in regulating gene expression in larval fat body.

766T **Exploring the functional limits and evolutionary patterns of shadow enhancers versus single enhancers** Jillian Ness[1], Yu Wang[2], Zeba Wunderlich[1,2] [1]Department of Biology, Boston University, [2]Department of Biomedical Engineering, Boston University

Developmental programs are guided by noncoding elements, called enhancers, that bind transcription factors and drive gene expression patterns. Most developmental genes in mammals, insects, and plants are regulated by sets of seemingly redundant enhancers that drive expression of overlapping spatiotemporal patterns. Separated by 100s – 10,000s bp, these multi-enhancer systems are termed shadow enhancers. The shadow enhancers can buffer genetic and environmental stress to drive gene expression patterns essential for normal development. Nevertheless, it remains unclear why shadow enhancer transcription factor binding sites are distributed across multiple enhancers rather than encoded within a single enhancer.

The primary objective of this project is to understand the fundamental limits of single enhancer regulation compared to multi-enhancer shadow regulation. It is conceivable that the various mechanisms shadow enhancers employ—such as distinct timing and levels of activation—could be encoded into a single enhancer in some circumstances.

We generated enhancer reporters in which the endogenous DNA between shadow enhancers is eliminated to make a 'squish' configuration of enhancers of two different developmental genes, *giant* and *Kruppel*. Using the MS2 system, we measured gene expression dynamics in live *Drosophila melanogaster* embryos. Surprisingly, we found little difference in RNA patterns, levels, and dynamics between the squish and endogenously spaced enhancers, suggesting the endogenous spacing is not needed for shadow function. Second, we tested the performance of squish enhancers against endogenous enhancers during heat stress and found no significant difference between *Kruppel* squish and endogenous shadow pair RNA levels and dynamics at temperature-matched conditions. Lastly, to understand the evolutionary pressures driving how shadow enhancers arise in animal genomes, we performed bioinformatic analyses to find evolutionary origins of developmental shadow enhancer sets. In these studies, we find duplication events and transposable elements appear to be a relatively small (25%) source of developmental shadow enhancer birth in *Drosophila,* while the contribution in mouse genomes is more appreciable (44%).

Taken together, these data indicate that combined, single enhancers can function comparably to distinct shadow enhancers, without compromising fidelity in a condition of stress. We can then probe the evolutionary dynamics and pressures that can create and maintain shadow enhancers in the genome to understand their pervasive role in animal development.

767T **Ribosome biogenesis: a new frontier in understanding the function of ribonucleoprotein Clueless in Drosophila** Aditya Sen[1,2], Ambar Rodriguez-Martinez[1,1,2], Sara K. Young-Baird[1], Rachel T. Cox[1] [1]Uniformed Services Univ., [2]Henry M Jackson Foundation for the Advancement of Military Medicine

Drosophila Clueless (Clu) and vertebrate CLUH are members of the conserved Clustered (CLU) mitochondrial ribonucleoprotein superfamily, essential for mitochondrial function. Both Drosophila Clu and yeast Clu1p bind mRNA, with CLUH preferentially binding mRNAs encoding mitochondrial proteins. Clu and CLUH associate with ribosomes at the mitochondrial outer membrane and it is unknown whether Clu's ribosomal association depends on mRNA. One open question is whether Clu's role at the ribosome is solely related to mRNA translation and stability, or whether it also plays a part in ribosome function and protein synthesis regulation. Previous studies, including work from our group, have shown that Clu homologs in *Saccharomyces*, *Drosophila*, and *Arabidopsis* associate with translation initiation factors. We also demonstrated that Clu interacts with ribosomal proteins from both the large and small subunits, and CLUH binds ribosomal proteins, as confirmed by mass spectrometry. Additionally, Clu sediments in higher-weight polysomal fractions, which shifts to lighter fractions when ribosomes are disrupted. Despite this, CLUH knockout or knockdown in mouse and human cell cultures does not impact overall protein synthesis levels but does alter the cell's metabolic state in an mTORC1-dependent manner, a key regulator of protein synthesis. This study aims to clarify Clu's association with ribosomes and its impact on ribosome function. Polysome profiling of *clu* mutant flies reveals a significantly reduced polysome-to-monomer ratio, contrasting with vertebrate cell culture studies. Moreover, *clu* mutant larvae show little puromycin incorporation, supporting the polysome profiling data and indicating a decrease in active protein synthesis. Clu binds ribosomal proteins independently of mRNA, suggesting a ribosome-specific role that does not require mRNA association. Although Clu is abundant in the cytoplasm, we found that it is also in the nucleus, where it associates with the nucleolar methyltransferase Fibrillarin, part of the small nucleolar RNA (snoRNA) complex involved in ribosomal RNA (rRNA) processing. Our data show that the decreased polysomes in *clu* mutants are not due to defects in rRNA processing, and ribosomal protein levels remain normal. Instead, *clu* mutants exhibit reduced abundance of both 40S and 60S ribosomal subunits. These findings strongly suggest that Clu plays a critical role in regulating ribosome biogenesis and overall protein synthesis.

768T **Coordinating stereotyped and stochastic patterns in the *Drosophila* eye** Alison Ordway, Caitlin Anderson, Robert Johnston Johns Hopkins University

During development, stereotyped patterning produces highly similar structures across individuals of the same genotype. In contrast, stochastic cell fate specification produces random patterns that are unique to each individual. How gene regulatory mechanisms are coordinated to generate stereotypical and stochastic patterns in the same tissue is poorly understood. Here, we address this question in the context of the developing *Drosophila* eye. The fly eye has a stereotypical array of photoreceptors that arises through a wave of morphogenesis driven by Hedgehog (Hh) signaling. Overlaid on this highly uniform structure is a random pattern of two R7 photoreceptor subtypes, controlled by stochastic ON/OFF expression of the transcription factor, Spineless (Ss). Here, we find that Hh regulates *ss* during stochastic R7 subtype patterning. *hh* mutants display a reduction in the size of the eye and the proportion of Ss^{ON} R7s. Loss of Hh in differentiating cells results in a loss of *ss* expression in precursors, whereas loss of the Hh receptor Patched (Ptc) derepresses Hh signaling and precocious expression of *ss* in undifferentiated cells. Our data suggest that Hh signals from differentiating photoreceptors to activate *ss* expression in precursor cells, similar to how Hh promotes morphogenesis of the fly eye. Cubitus Interruptus (Ci), an effector of Hh signaling, as well as Decapentaplegic (Dpp) and Homothorax (Hth), canonical downstream targets of Hh signaling do not appear to regulate *ss* expression, suggesting a non-canonical mechanism of action. We are now investigating alternative transcription factors that function downstream of Hh signaling to regulate stochastic *ss* expression. In summary, Hh signaling coordinates the generation of stereotyped eye morphology and stochastic R7 subtype patterning during development.

769T **GAGA Factor affinity for chromatin influences mitotic retention and gene expression in the early embryo** Annemarie E Branks[1], Marissa Gaskill[1], Hope Hawthorne[2], Kerstin Hurd[1], Melissa Harrison[1] [1]Biomolecular Chemistry, University of Wisconsin-Madison, [2]Biology, Massachusetts Institute of Technology

Changes in gene expression patterns drive developmental transitions. Once established, these gene-expression programs must be maintained to ensure normal development. Mitotic divisions are a major barrier to the maintenance of the gene-expression profiles required for cell-fate specification. During mitosis, the chromatin condenses, transcription arrests, and most transcription factors dissociate from the genome. Despite these substantial disruptions, the gene-expression profile of the cell is rapidly and robustly re-established following mitosis. A subset of transcription factors remains associated with mitotic chromosomes, suggesting that these proteins may function to maintain cell fate. Nonetheless, the functional relevance of mitotic retention of many transcription factors is unclear. To better understand how developmental programs are stably propagated across mitotic divisions, we leverage early *Drosophila* embryo development during which cell fates are specified as the nuclei undergo 13 rapid, synchronous mitotic divisions. We focus on GAGA factor (GAF), a transcription factor which promotes the establishment of both the active and silent genomes. GAF mitotic retention is evident at the pericentric heterochromatin during the rapid and synchronous early mitotic divisions, but at gastrulation GAF is no longer mitotically retained. GAF retention on euchromatin has been suggested to be instrumental in facilitating memory of the transcriptional state through mitosis. To understand the functional significance of mitotic retention, we identified protein domains important for GAF mitotic retention and a set of four serine residues that, when mutated, result in increased mitotic retention at both heterochromatin and euchromatin and continued mitotic retention of GAF into gastrulation. Our data suggest that increasing the affinity of GAF for chromatin promotes mitotic retention. Furthermore, we propose that maturation of the heterochromatin may limit GAF mitotic retention as development progresses. To determine the functional effects of increased GAF mitotic retention on gene expression, we generated mutations in GAF and performed RNA-sequencing of tightly staged single embryos. While the proposed role of GAF in mitotic memory would suggest that mitotic retention on euchromatin would promote GAF-target gene expression, we did not identify increased expression. Ongoing experiments using quantitative live imaging specifically test the effects of increased GAF mitotic retention on the dynamics of gene expression following mitosis. Together, our data indicate that developmental regulation of the affinity of GAF for chromatin regulates mitotic retention and we are uncovering how regulating mitotic retention affects gene expression and development.

770T **Developmental and Cell-type Specific Histone Gene Expression Patterns** Sierra Falcone[1], Leila Rieder[2] [1]Emory University, [2]Biology, Emory University

Histone protein concentrations help regulate the timing of rapid cell divisions in the early animal embryo. Misregulation is developmentally lethal: histone overexpression leads to extra or asynchronous cell divisions, while reduced histone expression leads to cell cycle arrest. As embryos develop, cell division dramatically slows. This change in the cell cycle leads to radically differing demands for histone transcripts and variable rates of histone gene expression. These cells must quickly alter their histone expression dynamics to match their cycling needs. Unequal demand for histone transcripts is perpetuated later in development as diverse cell types acquire novel proliferative potentials. How different cell types regulate identical sets of histone genes remains unclear.

I am leveraging existing tools available in Drosophila melanogaster, as well as the novel histone locus organization found in other Drosophila species to investigate mechanisms of histone gene expression. I will profile dynamic developmental histone gene expression from a single locus by creating a “barcoded” transgenic system in D. melanogaster to differentiate between histone genes and their transcripts. I will perform droplet digital PCR throughout embryonic development to probe for histone gene expression. I will also profile locus-specific expression of histone genes in tissues with varying histone requirements. I will be using SNV FISH to determine relative abundance of histone transcripts using Drosophila virilis, which carries two asymmetrical histone loci, and D. melanogaster-D. simulans hybrids. These experiments will elucidate critical mechanisms in gene family regulation by leveraging transgenic tools in D. melanogaster and underutilized genomes of non-melanogaster species.

771T **Comparing mechanisms of histone locus body (HLB) initiation and maintenance** Nicole Roos[1], Greg Kimmerer[2], Leila Rieder[2] [1]Biology, Emory University, [2]Emory University

Histone proteins are essential for compaction and regulation of the eukaryotic genome. Dysregulation of histone gene expression leads to aberrant development and lethality. In animals, genes encoding the histone proteins are commonly clustered. The histone locus body (HLB) is a nuclear body localized to histone loci and includes factors that regulate histone gene expression and transcript processing. It's unclear how histone genes are targeted by HLB factors during early embryogenesis and later in development. We seek to define mechanisms of HLB initiation and developmental maintenance to gain insight into how unique genes are targeted by factors for specialized regulation. Histone transgenes recruit HLB factors, allowing us to manipulate cis regulatory elements and determine the effects on HLB initiation and gene expression. To compare HLB initiation and maintenance mechanisms, we employ a transgenic system where we can remove regulatory cis elements during HLB maintenance. We will test whether HLB initiation can occur in non-embryonic cells through mitotic recombination experiments in wing discs and through transgenes that introduce cis elements required for HLB initiation. Overall, defining mechanisms of HLB initiation and maintenance illustrates how histone proteins are dynamically and developmentally regulated and in what contexts factors target these crucial genes for regulation.

772T **Transcriptional Regulation of Stochastic Cell Fate Specification in the *Drosophila* eye** Emma Steinson[1], Elizabeth Urban[2], Lukas Voortman[2], Spencer Zimmerman[3], Robert Johnston[2] [1]Biology, Johns Hopkins University, [2]Johns Hopkins University, [3]Stony Brook University

During development, cell fate decisions are often driven by lineage and signaling mechanisms. Generally, these mechanisms are robust, overcoming molecular noise to generate stereotypical patterns of cells. However, in some cell populations, stochastic cell fate specification harnesses molecular noise to diversify cell types to produce distinct proportions, but random patterns of cell types within tissues. Though stochastic cell fate specification is vital during development, the mechanisms that regulate these fate choices are poorly understood. Here, we investigate how cell-intrinsic mechanisms, specifically transcription and chromatin regulation, contribute to stochastic cell fate choices in the developing *Drosophila* eye. In the fly retina, R7 photoreceptors make a random, binary choice to express Rhodopsin 3 (Rh3) or Rhodopsin 4 (Rh4). The random pattern of R7 subtypes is controlled by the stochastic ON/OFF expression of a transcription factor called Spineless (Ss): Ss^{ON} R7s express Rh4 and Ss^{OFF} R7s express Rh3 in the adult retina. Although each R7 randomly chooses subtype fate, 67% choose Ss^{ON} R7 fate and 33% choose Ss^{OFF} R7 fate, generating consistent ratios of cell types yet random patterns. Our data suggest that stochastic expression of *ss* is controlled by the dynamic regulation of transcription and chromatin during development. An *early enhancer* activates expression of *ss,* and transcription opens the locus in photoreceptor precursors. Of these precursors, 67% express *ss* at high levels and 33% express at low levels. After transcription ceases, chromatin compacts in a subset of cells which eventually give rise to Ss^{OFF} R7s. In the cells that remain open, *ss* expression is re-activated by the *late enhancer* and these cells become Ss^{ON} R7s. We hypothesize that differential *spineless* expression in precursors drives the terminal Ss^{ON}/Ss^{OFF} R7 fate choice: high *ss*-expressing precursors become Ss^{ON} R7s and low *ss*-expressing precursors differentiate into Ss^{OFF} R7s. We developed reporters including the MS2 MCP system to monitor *ss* transcription initiation, elongation, and termination and the ParB/*parS* and LacI/*lacO* systems to track live chromatin compaction. Using these tools, we will investigate how variability in transcription and chromatin is harnessed to diversify cell types.

773T ***Cis*-element redundancy in *Drosophila melanogaster*.** Anthony Percival-Smith Biology, Univ Western Ontario

Testing the common assumption of phenotypic specificity (non-redundancy) of transcription factor (TF) function uncovered frequent redundancy of TF function, termed phenotypic nonspecificity. Eight out of nine TF dependent phenotypes are rescued by TFs other than the TF resident to the *TF* locus, non-resident TFs, at a frequency of 5-10%. The rescue is primarily due to functional complementation and not the expression of a downstream TF. Also, the TFs that rescue the phenotypes do not belong to similar TF families and do not recognize similar DNA-binding sites. Because distinct DNA-binding sites are recognized in phenotypic nonspecificity, there is the hypothetical expectation that the *cis*-regulatory elements used by non-resident TFs in a regulatory region are different from those used by the resident TF; simply put, the diversity of trans-acting factor recognition should be mirrored in a diversity of *cis*-acting regulatory sequences used. The rescue of depigmentation of the *dsx* null mutant abdomen by the expression of Doublesex female (DSX^F) is also rescued by the expression of Bric a Brac 1 (BAB1), Antennapedia (ANTP), Eyeless (EY) or Oddskipped (ODD). Analysis of epistasis suggests that all four TFs are epistatic to DSX^F and that ANTP, EY, ODD are epistatic to *bab* suggesting that these three TFs act after BAB or substitute for BAB function. BAB represses Yellow (Y) expression during the patterning of female abdominal pigmentation by binding to regulatory sequence in the *y* regulatory region. The cis-acting elements of the *y* regulatory region are well-characterized. Screening for repression of *y*::GFP reporter genes (provided by Thomas Williams) by BAB1, ANTP, EY or ODD, I found that of the three overlapping DNA fragments from the *y* regulatory region that are repressed by BAB1, two, the 1.1 and 0.9kb fragments, but not the 0.6Kb fragment, are repressed by ANTP. EY and ODD showed no repression of expression of these reporters suggesting that they work through elements outside of the *y* fragments tested. I have generated three 1kb DNA fragments fused to GFP: wild-type; no BAB DNA-binding sites; and no ANTP binding sites. The analysis of these GFP reporters will be presented at the meeting. The non-requirement of the *cis*-acting BAB binding sites of *y* (*cis*-element bypass) and the requirement of the *cis*-acting ANTP binding sites when ANTP is expressed would suggest that ANTP DNA binding sites in the regulatory sequence are being used to repress *y* expression indicating *cis*-element redundancy.

774T **Segregation Distorter and the Regulation of Satellite DNAs** Logan Edvalson[1], Xiaolu Wei[2], Amanda Larracuente[1] [1]Biology, University of Rochester, [2]Biomedical Genetics, University of Rochester

Meiotic drivers create genetic conflict by biasing their transmission to subsequent generations at a cost to the host organism. These conflicts arise in many taxa and may influence genome evolution, but little is known about how they exploit gametogenesis. Our lab studies the mechanism of the autosomal driver, Segregation Distorter (SD), in Drosophila melanogaster. SD is a sperm killer: SD/+ heterozygous males transmit SD to 95% of their offspring, whereas SD/+ females transmit SD fairly, to 50% of offspring. SD chromosomes are co-adapted gene complexes consisting of a driver and multiple enhancers, held in linkage by chromosomal inversions. SD causes a chromatin condensation defect in wild type chromosomes during spermatogenesis through an unknown mechanism. The drive target is a large block of tandem satellite DNA repeats called Responder (Rsp). The role of the Rsp satellite in SD drive is unknown. Our earlier work showed that Rsp behaves as a dual-stranded piRNA cluster and is expressed early in spermatogenesis. Because piRNAs play a role in establishing silent chromatin, we hypothesized that the Rsp-derived piRNAs might be involved in drive. We combine cytological, genetic, and genomic approaches to test this hypothesis. We find significantly fewer Rsp piRNAs associated with drive in some SD haplotypes. However, SD haplotypes with different inversions affect Rsp-derived RNAs differently. We hypothesize that SD haplotypes bearing different inversions may vary in their genetic enhancers leading to variation in mechanisms of drive. Further study of SD may provide insights into drive mechanisms and how satellite DNAs are regulated during spermatogenesis.

775F **The logic of transcriptional control in mitochondrial biogenesis** Fan Zhang[1], Annie Lee[2], Anna Freitas[3], Jake Herb[4], Zhe Chen[1], Hong Xu[1] [1]NHLBI, National Institutes of Health, [2]Uniformed Services University, [3]University of California, Berkeley, [4]Icahn School of Medicine at Mount Sinai

While mitochondria have their own genome that encodes core components necessary for oxidative phosphorylation and inner-mitochondrial translation, the majority of the over 1,000 mitochondrial proteins are encoded by the nuclear genome. The transcription regulations of mitochondrial biogenesis, the process of increasing mitochondrial content and activity, must be finely tuned to meet the cellular demands. Given the large number of mitochondrial genes and the dual genetic control over mitochondria, the current understanding of the transcription regulations of mitochondrial biogenies appears incomplete. We developed a genetic screening scheme in *Drosophila* eye to identify essential factors related to mitochondrial biogenesis. In a targeted RNAi screen covering all annotated transcription factors (TFs) in in the *Drosophila* genome, 77 TFs emerged as potential regulators of mitochondrial biogenesis. A regulatory network based on the chromatin binding profiles of these TFs was constructed to further elucidate the regulatory connections, hierarchy, and logic governing mitochondrial biogenesis. Contrary to the common belief that a few master regulators control all aspects of mitochondrial biogenesis, no single factor in the network covers most mitochondrial genes, or genes involved in a specific mitochondrial process. Instead, multiple transcription factors in the core layers of this multi-layered regulatory network show extensive connections and collectively control the expression of nearly all mitochondrial genes, whereas factors in the top layer may respond to cellular cues to modulate mitochondrial biogenesis through factors in underlayers. Besides the transcriptional regulation of mitochondrial biogenesis, our genetic scheme can also be applied to investigate other regulatory mechanisms, including post-transcriptional, translational, and post-translational regulations, which would lead to a more comprehensive understanding of the complex, multi-layered control of mitochondrial biogenesis.

776F **Relative enhancer-promoter configuration tunes transcriptional kinetics by modulating stability of the active state** Emilia A Leyes Porello[1], Robert Trudeau[1], Samantha Fallacaro[2], Louise Maillard[3], Emma Dreispiel Juan[1], Jiayi Wu[2], Mounia Lagha[3], Mustafa Mir[4], Bomyi Lim[1] [1]Chemical and Biomolecular Engineering, University of Pennsylvania, [2]University of Pennsylvania, [3]Institut de Génétique Moléculaire de Montpellier, [4]Children's Hospital of Philadelphia

The spatial relationship between enhancers and their cognate promoters in multicellular eukaryotes is complex. These regulatory elements can be separated by tens to hundreds of kilobases and exhibit variable relative positions, with enhancers located upstream, downstream, or even within introns of their target genes. This spatial flexibility enables sophisticated and precise control of gene expression in higher organisms. However, our understanding of how specific enhancer-promoter (E-P) configurations affect the kinetics of transcriptional activation remains limited. In this study, we investigate the regulatory mechanisms underlying E-P interactions and their impact on transcriptional activity in early *Drosophila* embryos. We employed reporter constructs that systematically modulate relative E-P positioning at distances ranging from 0 kb to 10 kb, coupled with the MS2/MCP live-imaging system for real-time quantification of transcriptional activity at the target locus. Our findings reveal that increasing E-P distance delays post-mitotic transcription activation and reduces mRNA production per nucleus throughout NC14. Strikingly – and in stark contrast to canonical understanding – we also find that placing the enhancer downstream of the promoter significantly impairs transcriptional output and stability of the active state. Quantifying the time spent in the active state (per nucleus) after initial activation reveals that the enhancer downstream subset marks a distinct population of diminished activity compared to the upstream enhancer group. We propose that this reduced active state stability reflects an instability of the transcriptional hub, potentially arising from RNA feedback. We propose a model where relative enhancer position to the promoter leads to varied degrees of nascent RNA integration to the transcriptional hub, alters the hub's stability, and affects transcriptional output. To further investigate this hypothesis, we introduced fluorophore-tagged Dorsal protein, a key activator of the *snail* enhancer-driven reporter genes and a primary component of the transcription hubs formed at these loci, and studied the correlation between hub behavior and transcriptional output at each transcriptionally active locus. These findings suggest there exist additional layers of regulatory control that depend on genomic context and provide new insights into the underlying mechanisms governing enhancer-mediated transcriptional activity.

777F ***trithorax (trx)* gene regulation of cardiac *Hox* gene expression and anterior-posterior patterning of the *Drosophila* heart tube** Sumaiya Islam, Md Sayeed Abu Rayhan, Shaad M Ahmad, Kristopher R Schwab Biological Sciences, Indiana State University

The *trx* and *trxG* genes encode conserved chromatin regulatory proteins that positively control developmental genes, such as the *Homeotic (Hox*) genes. In addition to patterning the anterior-posterior axis, *Hox* genes control the regional patterning of the developing heart. However, the precise regulation of cardiac *Hox* expression has yet to be explored. Previously, we have demonstrated *trx* is required for *abdominal-A (abd-A)* expression within the *Drosophila* embryonic dorsal vessel (heart tube). The loss of *abd-A* expression within the trx^{E2} null mutant causes a dramatic homeotic transformation of the posterior heart tube into an anterior fate, suggesting an essential role for *trx* function in heart development.

To better define cardiac *trx* activity, we have investigated unique hypomorphic *trx* strains for abnormal cardiac *Hox* expression and patterning. Unlike the severe amorphic trx^{E2} allele, the hypomorphic trx^{B20}, trx^{B21}, and trx^{B27} alleles provide a unique opportunity to study the cardiogenic function of *trx*. Although each hypomorphic allele is homozygous embryonic lethal, each allele can partially rescue embryonic lethality when crossed *in trans* to a *trx* amorphic allele. Whereas the trx^{B20} allele potentially disrupts the N-terminal protein interaction domain, the trx^{B21} and trx^{B27} alleles are predicted to inactivate the SET domain necessary for H3K4 methylation.

Our investigation of trx^{B20}, trx^{B21}, and trx^{B27} alleles has revealed interesting preliminary findings indicating unique cardiac developmental roles for the N- and C-terminal regions of the Trx protein. The trx^{B20} allele possesses a point mutation within the N-terminal Bromodomain and appears to be dispensable for *abd-A* expression since both the trx^{B20} null and trx^{B20}/trx^{E2} embryos have near normal posterior Abd-A levels and heart-proper patterning. Likewise, the trx^{B21} and trx^{B27} strains, which possess a SET domain truncation and point mutation, respectively, have near normal posterior Abd-A levels and heart-proper patterning. However, trx^{B21}/trx^{E2} and trx^{B27}/trx^{E2} transheterozygous embryos possess a moderate to severe reduction of *abd-A* expression, respectively, and abnormal heart-proper morphology, indicating insufficient *trx* activity necessary for proper Abd-A-mediated patterning in the posterior heart tube. Together, our preliminary results suggest that C-terminal SET domain activity and sufficient Trx dosage are necessary for proper cardiac *Hox* expression and patterning activity.

778F **Exploring the Role of miRNAs in Craniofacial Syndromes: A Genome-Wide Approach Using *Drosophila* Models** Manivannan Subramanian[1], Radhika Padma[1], Madhuri Kango-Singh[1,2,3,4], Amit Singh[1,2,3,4,5] [1]Department of Biology, University of Dayton, [2]Premedical Program, University of Dayton, [3]Center for Tissue Regeneration and Engineering at Dayton (TREND), University of Dayton, [4]Integrative Science and Engineering (ISE), University of Dayton, [5]Center for Genomic Advocacy (TCGA), Indiana State University

A dorsal selector gene *defective proventriculus* (*dve*), ortholog of human *SATB1*, is involved in a conserved mechanism of placement spacing of eyes on the heads. During organogenesis, GATA-1 transcription factor *pannier* (*pnr*) regulates *dve* to determine dorsal eye fate. Among various gene regulation mechanisms for *dve* expression, there is no information on transcriptional regulation mediated gene silencing by microRNA (miRNAs). miRNAs are the short hairpin like structure with 20-25bp which modulates the gene expressions post-transcriptionally by binding to 3'UTR of mRNAs. To discern genetic mechanism(s) regulating *dve* expression, we performed a forward genetic screen using a miRNA library in *Drosophila* eye and identified the miR-190 family as a genetic modifier. Gain-of-function (GOF) of *miR-190* results in increased eye size accompanied with increased expression domain of retinal determination genes, morphogenetic furrow marker, and reduced expression of negative regulator of eye fate markers like Wg, Hth. The increased eye size in *miR-190* GOF is due to increased cell proliferation with reduced cell death. Using bioinformatic analysis, we developed a miR-190-sensor which has *miR-190* binding sequence from Dve 3'UTR tagged to GFP. Targeted GOF of *miR-190* in domain specific manner eliminates GFP expression, which confirms *dve* as a target of *miR-190*. Regulation of *dve* by *miR-190* is conserved as *SATB1* also showed similar mode of regulation by miR-190a in humans and GOF of both *dve* and *SATB1*, rescues eye phenotypes of miR-190 in *Drosophila* models. We present a new mechanism of post-transcriptional regulation of *dve/SATB1* expression by *miR-190/miR-190a*. This study demonstrates that dysregulation of the *miR-190/SATB1* pathway leads to developmental defects in humans, including hypertelorism, which is characterized by an increased interocular distance and associated facial anomalies.

779F **Role of evolutionary conserved MicroRNA-190 in birth eye defects** Sunanda Yogi[1], Manivannan Subramanian[1], Madhuri-Kango Singh[1,2,3,4], Amit Singh[1,2,3,4,5] [1]Biology, University of Dayton, [2]Premedical Program, University of Dayton, [3]Center for Tissue Regeneration & Engineering (TREND), University of Dayton, [4]Integrative Science and Engineering (ISE), University of Dayton, [5]Center for Genomic Advocacy (TCGA), Indiana State University

Rare congenital eye disorders (birth eye defects) occur as partial or complete deformities in the eye. Sporadic and familial patients having poor vision exhibit range of developmental aberrations in the eye. The genes associated with birth eye defects play crucial roles in regulating networks of adjacent genes during eye development. Previous studies report that mutations in the short arm of human chromosome 11 affect the *Paired-box gene 6 (PAX-6)* and neighboring genes. The *Drosophila* homolog of *Pax-6* is *Eyeless (ey)*, studies on the gain-of-function of *ey* exhibit a "no-eye" phenotype. Hence, we aimed to understand how *PAX-6* function is regulated during eye development in the *Drosophila* eye model system. MicroRNAs (miRNAs) are non-coding RNAs that suppress the expression of target mRNAs either by its degradation or by inhibiting translation. Thus, miRNAs can regulate gene expression. In a forward genetic screen conducted in our laboratory, we identified a novel miRNA-190, as a potential candidate to regulate *ey*. Further bioinformatic analysis revealed that miRNA-190 is conserved in humans. Therefore, we sought to understand the role of miRNA-190 in regulating *ey* using the UAS-Gal4 bipartite tool and a miRNA-190 sensor approach. We checked its effect on eye development by examining retinal determination and differentiation (RD) gene markers. To validate this interaction, we performed both gain-of-function and loss-of-function studies of *ey* in the background of miRNA-190 expression. These studies elucidate mechanistic basis of *ey*-miRNA-190 regulation and in turn tune the genetic circuitry involved in the congenital etiology of rare eye defects seen in retinopathies and craniofacial defects.

780F **Spatiotemporal Regulation of Early Neurodevelopmental Gene Expression in *Drosophila* Using Single-Cell Multiome Sequencing** Priyanshi Borad[1], Vanessa Avila[2], Anna Makridou[3], Eva Martou[3], Kelli Fenelon[2], Theodora koromila[4] [1]Biology, The University of Texas at Arlington, [2]The University of Texas at Arlington, [3]Aristotle University of Thessaloniki, [4]The University of Texas Arlington

Neurodevelopmental disorders, such as ADHD, are often rooted in early brain development disruptions, where transcription factors (TFs) play essential roles in cell fate decisions. These regulatory mechanisms are evolutionarily conserved between *Drosophila* and humans, making *Drosophila* a valuable model for studying neurogenesis. From our previous study, two TFs, Odd-paired (Opa) and Ocelliless (Oc), analogous to mammalian ZIC3 and OTX1/2, respectively, co-regulates gene expression in embryonic brain regions. Preliminary in situ results can confirm the expression patterns of target genes and enhancer activity in the Opa-Oc co-regulated regions, providing a spatial map of potential regulatory zones. We specifically examined enhancers near brain genes such as empty spiracles *(ems*) and twin of eyeless (*toy)*, expressed in early *Drosophila* embryogenesis. Utilizing live imaging techniques, we can directly visualize the role of *toy*, an early brain marker, in real time. This methodology provides high-resolution spatial and temporal data on the activity of TFs at single-cell level. By tracking the cellular localization, expression pattern, interactions of *toy* and associated TFs provides deeper insights into the molecular mechanisms driving brain development. This approach also facilitates the identification of key cellular events such as lineage specification, differentiation and synaptic maturation in a spatiotemporal context. Considering the abundance of regulatory elements influencing *ems* during stages before and just after gastrulation, CRISPR/Cas9 was used to investigate endogenous gene expression. Using single-cell multiomics, we aim to profile chromatin accessibility and transcriptional states within individual cells across developmental timepoints. This dual-omics approach allows us to capture a comprehensive regulatory landscape, linking enhancer activity and TF binding directly to gene expression profiles, particularly in regions expressing neurogenic genes such as *ems*, and *toy*. By correlating chromatin accessibility with transcriptional outputs, we can capture transient regulatory interactions that define neurogenic domains. Through elucidating the molecular dynamics of brain development, our findings will contribute to a deeper understanding of the genetic architecture involved in early neurodevelopment, offering a window into the origins of disorders like ADHD.

781F **Analysis of cis-regulatory sequences from the midline locus reveals a bifunctional regulatory element that is directly regulated by BMP signaling and mediates non-additive interactions with an adjacent enhancer.** Laura Nilson[1], Kelvin K Ip[2], Baptiste Rafanel[3] [1]McGill University, [2]Department of Biology, McGill University, [3]Institute of Molecular Biotechnology, Austrian Academy of Sciences

Complex gene expression patterns can emerge in a developing tissue through a combination of positional cues which converge on the cis-regulatory modules (CRMs) of their downstream target genes. Multiple CRMs can act additively, where each contributes one component of the final expression pattern, or non-additively, e.g. to refine expression boundaries. As a patterning model, we study the Drosophila follicular epithelium, where input from multiple localized positional cues is integrated to generate the patterned expression of anterior and posterior fate determinants. Here we focus on the regulation of the posterior follicle cell (FC) fate determinant *midline (mid)*, which is repressed in anterior FCs by BMP signaling. Our characterization of a putative *mid* CRM (named mid-in) reveals that BMP signaling directly regulates CRM expression and also mediates non-additive interactions between CRM sub-regions.

The mid-in CRM drives reporter expression in a pattern that largely recapitulates that of endogenous *mid* and is repressed by BMP signaling in anterior FCs. Mutation of two putative binding sites (MBSs) for the Dpp signaling effector MAD/Medea results in ubiquitous FC expression of mid-in, suggesting that BMP signaling directly represses the enhancer activity of mid-in.

The full-length mid-in (mid-in.full) can be subdivided into two contiguous portions: mid-in.2, which contains the MBSs and drives expression in posterior FCs, and mid-in.1, which drives expression in all FCs. The ubiquitous expression implies that mid-in.1 contains a general FC enhancer which is normally suppressed in the context of mid-in.full. This suppression requires the intact MBSs, suggesting that the mid-in.1 enhancer is silenced by BMP signaling in the mid-in.full context.

Although the MBSs mediate repression in the context of mid-in.full, they are instead required for expression of mid-in.2, suggesting that the MBSs define a bifunctional regulatory element that can act as an enhancer or silencer depending on the local sequence context. Unexpectedly, mid-in.2 is repressed by ectopic BMP signaling, suggesting that the MBSs can activate or repress expression depending on signaling dosage.

Our data show that mid-in enhancer activity is directly regulated by BMP signaling via the MBSs, that these MBSs are required for suppression of a mid-in.1 enhancer in mid-in.full, and that the MBSs define a bifunctional regulatory element whose functionality depends on the sequence and signaling context.

782F **Understanding the gene regulation dynamics in embryonic heart development** Shiva Abbasi[1], Evangelia Chrysostomou[2], Rie V Conley[1], George Tegousis[3], Theodora Koromila[4] [1]Biology, The University of Texas at Arlington, [2]Biology, University of Thessaloniki, [3]University of Thessaloniki, [4]The University of Texas at Arlington

Embryonic heart development is a complex process driven by the precisely timed and sequential interactions of key regulatory proteins. The rapidly developing *Drosophila* embryo provides an excellent model for studying the dynamic co-regulation of significant transcription factors (TFs) such as Odd-paired (Opa) before and after gastrulation. Opa/ZIC3 and Twi/TWI1 have been observed to co-expressed during cellularization. Is there a role for Opa and Twi complex, in orchestrating the expression of cardiac genes, including early and late enhancers? While Opa›s involvement in embryo development after gastrulation is well-documented, such as Opa activity as a timing factor after gastrulation, its specific role in the early embryo before gastrulation remains elusive. We hypothesize that Opa/ZIC3 regulates heart specific genes before gastrulation. By ChIP-seq and ATAC-seq meta-analysis, we analyzed the role of Opa in cardiac genes, both before and after gastrulation. Additionally, in situ staining and RNA-seq meta analysis showed the role of Opa in cardiac gene expression pattern and level. Using advanced techniques like live-imaging super-resolution microscopy and single-cell multi-omic analyses, our study provides deeper insights into the regulatory mechanisms by which transcription factors expressed during cellularization direct heart cell specification in early embryogenesis. Funding: STARs program UTA

783F **Compartmentalised alternative splicing of *Down Syndrome Cell Adhesion Molecule* (*Dscam*) gene in the brain is conserved between *Drosophila* and honey bees** Anna K Lassota[1], Matthias Soller[1,2] [1]School of Biosciences, College of Life and Environmental Sciences, University of Birmingham, [2]Division of Molecular and Cellular Function, School of Biological Sciences, University of Manchester

Alternative splicing is a major mechanism to generate molecular diversity of proteins from a limited number of genes. *Drosophila Down Syndrome Cell Adhesion Molecule* (*Dscam*) is the record holder in alternative splicing diversity with 38,016 different proteins made from one gene. Initial models proposed that exon selection occurs stochastically via RNA secondary structure mechanism. However, recent studies show that deleting the common base-pairing sequence does not alter splicing patterns and that base-pairing is coincidental because G can also pair with U. Furthermore, a polar effect favouring the inclusion of exons closer to the common sequence is not observed. Since *Dscam* alternative splicing can adapt to generate isoforms with higher affinity for pathogens in mosquitos and changes in honey bees after learning, exon selection seems regulated rather than stochastic in differentiated cells.

To determine whether variable exon inclusion is stochastic, we used transgenic reporter lines in *Drosophila* with +1 and -1 nucleotide modifications to analyse the inclusion patterns of specific variable exons. We found that variable exon expression is not entirely stochastic and contains a deterministic aspect in larval brains. Here, exon inclusion appears in specific patterns challenging the stochastic model of exon selection. Through RNA *in situ* hybridization experiments in honey bee mushroom bodies, we observed compartmentalised inclusion of *Dscam* variable exons. However, inclusion levels and locations vary between individuals arguing that *Dscam* alternative splicing is altered upon experience in honey bees.

784F **Investigation of the Jak-Stat Pathway** Hanna Landguth[1], Xiaoyu Kang[2], Bruce Edgar[2] [1]Huntsman Cancer Institute, [2]Hunstman Cancer Institute

The Jak-Stat Pathway is crucial as it plays central roles in immune and inflammatory responses, regeneration, wound healing, and human diseases such as auto-immune disorders, inflammatory bowel diseases (IBD), and cancers. Our research aims to investigate the function of downstream target genes of the Jak-Stat Pathway in *Drosophila* midgut regeneration including socs36E, zfh1, chinmo, and other potential target genes. *Drosophila* is an excellent research model because the Jak-Stat pathway is highly conserved in *Drosophila* but much simpler. For example, in humans, there are more than 30 cytokines, whereas in flies there are 3, identified as unpaired (upd) 1,2, and 3. Similarly, there is one cytokine receptor, Domeless (Dome); one Jak, Hopscotch (Hop); and one transcription factor STAT, Stat92E. Our previous study shows that the Jak-Stat pathway is essential for differentiation in the *Drosophila* gut and contributes to cell proliferation. Although those studies showed that the JAK-STAT pathway has some crucial function in gut regeneration, it is still unknown what effects downstream control of those processes. Here, we want to investigate the downstream targets of the Jak-Stat pathway to find the effector that controls stem cell proliferation and differentiation. zfh1 and chinmo are genes that has been identified by previous study that are Jak-Stat target genes. However, their function in the gut regeneration is still unknown. To investigate that, we plan to manipulate those genes using TARGET esg Flip Out System, which is a cell lineage tracing system under the control of temperature, with gene overexpression or knocking down (RNAi). From there, we will count phospho-histone H3 positive (PH3+) cells for mitosis as well as using cell markers to identify the cell type. By studying the downstream target genes of the Jak-Stat pathway in *Drosophila,* we may contribute to the clinical study on JAK-STAT pathway, for example, reducing the side effects of existent JAK inhibitor or STAT inhibitor by providing a more downstream and specific target.

785F **Mito-Nuclear Signals in Mitochondrial Biogenesis and Cellular Stress Responses** Shane Grele, Fan Zhang, Hong Xu National Institutes of Health

The process by which cells sense and respond to mitochondrial abundance in order to meet developmental and tissue-specific energy demands remains largely unknown. Prior work to characterize transcriptional regulation of mitochondrial biogenesis includes a modified RNAi screen performed in the developing eyes of *D. melanogaster* targeting 638 annotated transcription factors (TFs), which identified 77 enhancers and 20 suppressors, with published ChIP data allowing for construction of a regulatory network. For these genes, RNAi in conjunction with MitoXhoI, a restriction enzyme targeting mitochondrial DNA (mtDNA), reduced eye size in a synergistic manner, suggesting their involvement in mitochondrial biogenesis in response to mtDNA deficiency. Furthermore, mtDNA deficiency triggers the compensatory expression of nuclear-encoded mitochondrial genes. The creation of an endogenous reporter construct in *Drosophila* has allowed for subcellular imaging of the midgut monolayer epithelium to be used in demonstrating this effect. Treatment with MitoXhoI, as with other mitochondrially targeted restriction enzymes, induced endogenous expression of nuclear-encoded mitochondrial genes, further demonstrating mitochondrial biogenesis in response to mtDNA deficiency. Through testing the subcellular localization of different tagged reporter constructs *in vitro* in both *Drosophila* and mammalian cells in response to MitoXhoI, we expect to reveal conservation of gene activity. Using a similarly modified RNAi screen, along with subcellular midgut imaging, we plan to assess candidate factors involved in the mechanisms behind the compensatory expression of nuclear-encoded genes in response to mitochondrial stress. Finally, we expect to use RNA sequencing to further assess transcriptional changes in response to mtDNA deficiency.

786F **Pathological Contributions of Abnormal tRNA-derived Fragment Populations** Lucia I. Vilchez, Saathvika Rajamani, Edward Dubrovksy Biological Sciences, Fordham University

Emerging evidence suggests that transfer RNA (tRNA)-derived fragments (tRFs) play a significant role in the molecular mechanisms underlying some human diseases. These biomolecules are a novel subclass of small noncoding RNAs that are generated by the cleavage of pre- and mature tRNAs. While their biological functions are still not well defined, studies have suggested that these molecules may be involved in gene regulation through incorporation into the RISC complex and interaction with argonaute (Ago) proteins. Using the *Drosophila* model, we have identified differentially expressed tRFs through small RNA-sequencing in flies with impaired tRNA processing. Specifically, these flies lack or express a mutant isoform of the RNase Z endoribonuclease responsible for the cleavage of the 3' trailer of pre-tRNA molecules. Using various bioinformatic analyses, we have identified groups of tRFs that have altered expression as a result of RNase Z mutation or knockout. Further, we have identified putative target genes of upregulated tRFs. To determine if these dysregulated genes were enriched for functional categories, we looked at gene ontology information, which revealed enrichment categories for synaptic transmission, neuronal function, and neurodevelopment. Consistently, we have previously observed that flies harboring the mutant RNase Z allele (Thr494Ile) exhibit reduced lifespan, developmental delays, and neurological abnormalities. These phenotypes mirror what is seen in human patients with this mutant allele, suggesting a possible contribution of these molecules to RNase Z-associated pathology. The relationship between tRFs and disease points to a potential role for these fragments as diagnostic and prognostic biomarkers as well as therapeutic targets.

787F ***Polycomb (Pc)* and *Pc Group (PcG)* genes repress *trithorax (trx)*-mediated *Hox* expression and cardiac patterning within the *Drosophila* heart tube.** Md Sayeed Abu Rayhan[1,2,3], Sumaiya Islam[1,2,3], Adam Farmer[1], Shaad Ahmad[1,2,3], Kristopher Schwab[1,2,3] [1]Biology, Indiana State University, [2]The Rich and Robin Porter Cancer Research Center, [3]The Center for Genomic Advocacy

The PcG proteins antagonize the activity of the Trithorax (Trx) and the Trithorax group (TrxG) by repressing the transcription of important developmental genes such as the *Hox* transcription factors. We have previously identified *trx* as a positive regulator of *Hox* expression and anterior-posterior patterning within the *Drosophila* embryonic dorsal vessel (heart tube). The wildtype heart tube is organized into two distinct anatomical regions: the narrow aorta and the wide heart-proper regions. *trx* maintains the expression of *abdominal-A (abd-A)* in the posterior heart tube which is necessary for heart-proper patterning, while *Pc* antagonizes *trx* activity by repressing *abd-A* expression within the anterior heart tube maintaining the aorta fate.

To determine the precise roles for *trx* and *Pc* in *Drosophila* heart development, a *trx, Pc* recombinant strain possessing both amorphic alleles was generated to evaluate the interaction of these genes. Remarkably, the homozygous *trx, Pc* null embryos recapitulated the *trx* phenotype consisting of the absence of *abd-A* expression and heart-proper patterning. This data suggests that cardiac *Hox* activity requires *trx*-mediated activation in the posterior heart-proper region, whereas *Pc* function is only necessary to represses ectopic *Hox* expression within the anterior aorta.

Additionally, other PcG may also be modulating cardiac *Hox* gene activity. *PcG*-mediated repression of gene expression is mediated by several complexes, including the Polycomb-repressive complex 1 (PRC1) and PRC2. Our screen of *PRC1* mutant strains for aberrant cardiac *Hox* expression and patterning has successfully identified the *Sex combs on midleg (Scm*) gene as necessary for anterior cardiac *abd-A* repression since the *Scm* null heart tube phenocopies the Pc phenotype. In contrast to *Pc* and *Scm*, our screen of several PRC2 genes indicate PRC2 activity may be dispensable for *abd-A* repression since *abd-A* expression remains restricted to the posterior heart-proper similar to the wildtype phenotype. These preliminary findings identify distinct roles of *PcG* regulation in embryonic heart development suggesting that PRC1 activity, rather than PRC2 function, is necessary for normal *Hox* repression and subsequent patterning.

788F **Bruno 1 isoform-specific function in D. melanogaster indirect flight muscle (IFM)** Aaron Morgan, Maria Spletter, Jenna DeCata University of Missouri Kansas City

CUGBP Elav-like Factor (CELF) family proteins are important fiber-type specific splicing regulators in muscle and are implicated in diseases such as Myotonic Dystrophy Type 1 (DM1). While vertebrate genomes encode six CELF family members which are partially redundant, a single CELF homolog Bruno1 (Bru1, aret) has been shown to be a key RNA regulator during development of indirect flight muscle (IFM) in *D. melanogaster*. We previously showed that Bru1 regulates cytoskeletal rearrangements supporting myofibrillogenesis, is necessary for a maturation switch in the splicing of IFM-specific isoforms, and regulates both sarcomere growth and myosin contractility. Bru1 itself is alternatively spliced, and structural analysis identifies distinct N-terminal regions on longer isoforms as well as a novel isoform that has only two RNA-recognition motif (RRM) domains. Although vertebrate CELF proteins are also alternatively spliced, little is known about isoform-specific CELF function. Here we show via transcriptomics and Western blot that multiple Bru1 isoforms are expressed in IFM. We identify isoform-specific phenotypes and transcriptomic signatures, suggesting a differential requirement for different Bru1 isoforms during IFM myogenesis. To identify direct RNA targets of specific Bru1 isoforms, we performed enhanced UV-crosslinking immunoprecipitation (eCLIP) experiments. GFP-tagged isoform-specific UAS constructs were driven selectively in IFM with Flightin-Gal4 and compared to a UAS-GFP as well as an endogenous CRISPR Bru1-eGFP tagged line. We further evaluated isoform-specific protein interactors using immunoprecipitation mass-spectrometry. Our data provide insight into Bru1 direct targets in IFM and provide the foundation for future biochemical experiments using EMSA or RNA Bind-N-Seq (RBNS) experiments to measure Bru1 isoform-specific binding affinity. Our data are the first demonstration of CELF protein isoform-specific function, expanding the current model of the RNA regulatory network in muscle and advancing our understanding of CELF protein function in muscle development and disease.

789F **The Role of Heat Shock Protein 70 (HSP70) in Stress Adaptation and Environmental Resilience of Catfish** Faith O Ayoade, Opeyemi A Oladejo, Abel O Oguntunji Animal Science and Fisheries Management unit, Bowen University, Nigeria

The Heat Shock Protein 70 (HSP70) gene plays a crucial role in mediating stress responses in fish, especially in species of aquaculture significance like Catfish. Serving as a molecular chaperone, HSP70 is essential for protein folding, cellular stabilization, and mitigating damage under stressors such as temperature fluctuations, hypoxia, and pathogen exposure. This study examines the functional pathways and genetic contributions of HSP70 to stress resilience, leveraging gene set enrichment analysis to highlight its pivotal roles in “Protein processing in the endoplasmic reticulum” (FDR = 1.5E-03; Fold Enrichment = 61.8) and “Spliceosome” (FDR = 4.5E-02; Fold Enrichment = 44.4) pathways. These findings underscore the involvement in protein homeostasis and RNA processing, which are essential for cellular stability in stress adaptation. In catfish, HSP70 expression is responsive to thermal and salinity stress, indicating its potential as a biomarker for selective breeding, aiding in the development of resilient fish strains.

This research reveals that while HSP70 upregulation supports environmental resilience, excessive activation may impact growth and reproduction, necessitating a balanced approach to stress management in aquaculture. Furthermore, the high homology of the gene with other Siluriformes positions HSP70 as a target for breeding programs aimed at enhancing genetic adaptation. By linking HSP70-associated pathways to environmental adaptation, this study advances sustainable aquaculture practices, fostering resilience against environmental stressors and promoting the health and productivity of fish stocks under dynamic conditions.

790F **Transcription of OVO target genes are dependent on OVO binding and can overcome repressive heterochromatin** Lorielle Raab[1,2], Leif Benner[1], Brian Oliver[2], Leah F Rosin[1] [1]NICHD, National Institutes of Health, [2]NIDDK, National Institutes of Health

The Drosophila female germline specific transcription factor OVO has recently been shown to transcriptionally regulate numerous well characterized maternal-effect genes that are required for embryonic development. OVO was found to bind at transcriptional start sites genome-wide and this binding positively influenced gene expression. OVO binding was also highly associated with open chromatin genome-wide, however, it is still unclear exactly the mechanism through which OVO regulates transcription and if open chromatin is a consequence of OVO binding or simply the regions to which OVO can bind genome-wide. To address this, we took two OVO germline target genes, *bicoid* (*bcd*) and *oskar* (*osk*), and placed the OVO bound genomic fragment (OVO enhancer fragment) upstream of a basal promoter and H2A-3xV5 reporter gene. We also made a version of the same OVO enhancer fragments, but we deleted all the significant OVO DNA binding motifs within. We placed these reporters in a well-established heterochromatic region on the X chromosome and found that both the *bcd* and *osk* reporters were able to drive expression of the H2A-3xV5 specifically in germ cells of the ovary, which is where OVO is expressed. In contrast, the enhancer fragments without the OVO DNA binding motifs lacked detectable germline expression. This result indicates that OVO binding is required for enhancer activity and can overcome the local repressive chromatin state in this region. We are currently conducting experiments to test the relative accessibility between the enhancer fragments that contain or do not contain OVO DNA binding motif through ATAC-seq, to determine if OVO binding can directly influence an open chromatin state for these enhancers. We are also interested in determining what other proteins are in complex with OVO at these regions to better understand how OVO directs female germ cell specific transcription.

791S **Zelda as a Pioneer: Coordinated Activation of Minor-wave Gene Pair** Ram Wagle, Alicia Zhu, Mirabelle Moore, Christine Rushlow Biology, New York University

Early embryonic development requires a transition from maternal to zygotic control, driven by zygotic genome activation (ZGA). A key feature of the earliest genes activated during ZGA, known as minor-wave genes, is a conserved regulatory motif comprising a TATA box at -30 and two or more Zelda (Zld) binding sites (CAGGTAG) just upstream of TATA. This arrangement of Zld binding sites in the 5' regulatory regions is conserved across Drosophilid species. Similarly, zebrafish minor-wave genes exhibit a comparable motif, with pioneer transcription factor (PTF) binding sites near a TATA box. We hypothesize that this spatial organization facilitates interactions between PTFs and TATA-binding protein (TBP). Notably, about half of these genes are organized in pairs, transcribed in either the same or opposite directions.

To explore this regulatory mechanism, we investigated Zld binding sites in the activation of *Elba1* and *Elba3*, a gene pair in opposite orientation separated by approximately 3 kb. Using CRISPR/Cas9, we systematically mutated the two Zld sites in *Elba1* (*Elba1* double mutant-*dm*), the two strong sites in *Elba3* (*Elba3* double mutant-*dm*), and all four sites together (*Elba1/3* quadruple mutant-*qm*).

Using RNA *in situ* hybridization, we show that *Elba1* and *Elba3* mRNAs are absent in *zld*$^{-}$ and *Elba1/3-qm* mutants, indicating that Zld binding sites are essential for activation. In *Elba1-dm* and *Elba3-dm* mutants, expression is partially reduced, suggesting that Zld sites in one gene enhance expression of the other, likely via chromatin looping or shared transcriptional hubs. Moreover, rescued expression displayed anterior bias, implicating Bicoid (Bcd) and Hunchback (Hb) in activating *Elba1* and *Elba3*. However, this activation depends on Zld, as expression is abolished in *Elba1/3-qm*. In support of this, Bcd and Hb binding sites were recovered between Zld binding sites in the regulatory regions of *Elba1* and *Elba3*. Future experiments focused on the roles of these factors will shed light on the interplay of Zld and co-regulators in transcriptional regulation of these genes.

Altogether, our findings underscore cooperative role of Zld in pioneering chromatin accessibility and facilitating enhancer-like interactions between neighboring genes. This work provides insight into the transcriptional regulation of minor-wave genes and conserved regulatory mechanisms across species.

Keywords: Pioneer factor, Zelda, Minor-wave genes, *Drosophila*.

792S **Identification of a candidate *akirin* enhancer sequence** Alyssa DeSantis[1], Diana Esmaeilzadeh[1], Georgette-Vanelle Wandji[2], Scott J Nowak[1] [1]Molecular and Cellular Biology, Kennesaw State University, [2]Biology, Boston University

Akirin, a small nuclear protein with conserved function across eukaryotes, is a critical determinant in the development of functional, robust cardiac and skeletal patterning and musculature. Akirin serves as a transcription cofactor by acting as a link between transcription factors such as Twist. Akirin uses chromatin remodeling complexes to ensure that Twist functions appropriately during transcription. If Akirin function is impaired, the resulting muscle patterning and structure is greatly impacted. We have identified a short sequence within the first intron of *akirin* that is highly conserved among closely related *Drosophilid* species. We are evaluating this sequence for possible promoter or enhancer activity. This evaluation is accomplished utilizing a variety of *in vivo* and *in vitro* techniques, both in live *Drosophila* embryos, as well as in cultured S2 cells. We have determined that a likely candidate enhancer sequence does indeed occur within this conserved element and are investigating a number of candidates that regulate this particular DNA sequence for *akirin* expression.

793S **Determining the role of Myc in *Drosophila* histone gene expression** Juliana Christie, Casey Schmidt Lafayette College

Histones play a crucial role in DNA packaging: they serve as a spool for DNA to wrap around to fit into a compact nucleus. Because histones directly interact with DNA, their levels are tightly regulated in cells, and the consequences of misregulation are dire. Excess histones are toxic to cells, whereas a paucity of histones leads to developmental arrest. Histone mRNA expression and processing is controlled by the histone locus body (HLB) to achieve these regulatory requirements. The HLB is a complex nuclear body composed of various proteins, including the core structural component, Mxc. Although there have been several screens to identify new HLB members, some HLB components have been discovered serendipitously. For example, the transcription factor Myc was found to localize to the HLB in several *Drosophila* tissues. To elucidate Myc's role in histone gene regulation, we analyzed publicly available ChIP-seq datasets. We consistently found that Myc targets the H2A-H2B promoter region of the *Drosophila* histone gene array. We also observed that Myc localizes more strongly during interphase than mitosis, likely because histones are primarily expressed in S-phase of the cell cycle. To follow up on this preliminary data, we will assess histone expression in embryos when *myc* is both knocked down and overexpressed. Given that Myc's localization to the HLB correlates with the phosphorylation of Mxc, we will also examine *cyclinE* mutants to determine if Myc HLB localization is dependent on Mxc phosphorylation. In addition, we will map the Myc binding site in the H2A-H2B promoter region using gel shift assays. Together, these experiments will clarify the role of Myc in histone gene expression, and give insight into how transcription factors collaborate to promote coordinated gene expression.

794S **Uncovering Sage's Collaborative Role in Salivary Gland Gene Regulation** Nathaniel Laughner Johns Hopkins University

Transcription factor (TF) networks are complex intricate systems that require coordination between multiple TFs to drive organogenesis and tissue-specific gene expression programs. Within the model system of the Drosophila embryonic/larval salivary gland, the basic helix-loop-helix TF known as Sage interacts with two other TFs - Fork head (Fkh) and Senseless (Sens) - to regulate expression of genes encoding secretory cargo proteins, the enzymes that modify these cargos, pro-apoptotic factors, and, likely, factors regulating overall organismal metabolism. To elucidate the mechanisms of TF interactions and gene regulation, we are utilizing the embryonic salivary gland to uncover how these three TFs interact with each other and with their endogenous targets to establish and maintain salivary gland-specific functions. First, we are identifying the full suite of genes that are bound by each factor in the embryonic salivary gland using tissue-specific Chromatin Immunoprecipitation and Sequencing (ChIP-Seq). Second, we are identifying SG-expressed genes that are affected by the loss of each TF using microarrays and single cell RNA sequencing (scRNASeq) to reveal not only how binding and gene expression are related but to also learn the range of functions under the control of each TF. By studying the correlations between TF binding site configurations and TF-dependent gene expression, we will develop and test models for coordinate gene regulation in the context of a functional organ. Understanding the molecular details of how Sage, Fkh and Sens coordinate binding and gene regulation will illuminate how tissue-specific gene expression is achieved in this system and will provide paradigms for how transcription factors network in more complex organisms. We are additionally interested in learning if Sage plays a more direct role in controlling metabolic functions and, if so, identifying its relevant downstream targets.

795S **Exploring Overlapping Cis-Regulatory Elements in the Regulation of *ftz* and *Scr* in *Drosophila melanogaster*** Kristen Au, Leslie Pick Entomology Department and Graduate Program in Molecular and Cell Biology, University of Maryland

Embryonic development relies on precise gene regulation directed by cis-regulatory elements (CREs). However, much remains to be learned about the role of CREs in gene regulation, even in the well-studied model system *Drosophila melanogaster*. Enhancer choice and specificity are complex and still poorly understood, especially in cases where CREs are located between adjacent genes. This complexity is evident for the segmentation gene *fushi tarazu* (*ftz*), where distinct CREs drive its expression, despite some being located in shared intergenic regions with the neighboring gene Sex combs reduced (*Scr*). Studies using rescue and reporter transgenes have identified multiple CREs that regulate *ftz* expression. However, an XbaI genomic fragment within the *ftz* locus, located between other CREs, was found to be dispensable for rescuing the mutant phenotype (Hiromi et al., 1985). Further investigation revealed that this ‹dispensable region,› while not essential for *ftz* expression, directed transgene expression in a pattern resembling that of *Scr* (Gindhart et al., 1995). Specifically, a XbaI/HindIII fragment within the 'dispensable region' drove *lacZ* expression in the anterior midgut, a domain consistent with *Scr*'s expression in the visceral mesoderm. This suggested the intriguing possibility that a region embedded within *ftz* CREs regulates the expression of *Scr*. We asked whether precisely deleting this region via the CRISPR/Cas9 system through homology directed repair will decrease midgut expression of *Scr* and/or influence *ftz* expression. If this region is truly dispensable for *ftz* regulation, then its deletion should not affect *ftz* expression. Previous work in our lab suggests that an insulator element within the 'dispensable region' may prevent crosstalk between *ftz* and the adjacent region. We hypothesize that if the XbaI-HindIII region contains an insulator element, its deletion will result in ectopic *ftz* expression in the anterior midgut, mimicking *Scr*-like patterns. In addition, Scr may be expressed in ftz-like stripes. These experiments will deepen our understanding of the mechanisms that maintain gene expression and contribute to our broader knowledge of genome organization and regulatory networks in development.

796S **The regulation and physiological role of transcription factor REPTOR in *Drosophila* fat bodies** Yuwei Sun, Ting Miao, Kerui Huang, Yuwei Sun, Norbert Perrimon Department of genetics, Harvard Medical School

An enormous amount of groundbreaking work has gone into understanding the mechanisms and relationships of obesity and diabetes, while *CREBRF* (CREB3 Regulatory Factor) could be one of the noteworthy factors. Previous studies have shown that *CREBRF* variants are strongly associated with increased odds of obesity but decreased odds of diabetes, yet how it regulates lipid storage, body mass, and metabolic homeostasis is largely unknown.

Here we focused on the role of the fly ortholog of *CREBRF*, *REPTOR,* in *Drosophila* fat bodies. Our current findings show that *REPTOR* positively regulated lipid storage, with overexpression leading to increased lipid accumulation in the fat bodies, possible through upregulation of *de novo* lipogenesis pathway. Additionally, *REPTOR* activity in the fat bodies was observed to increase when fed on high-sugar diet (HSD), while starvation led to *REPTOR* inactivation. Intriguingly, increased fat content in the fat bodies under HSD is completely blocked by knockdown of *REPTOR*. Meanwhile, we found that the flies were intolerant to sugar content when knocking down REPTOR in the fat bodies. In addition, the key genes in sugar sensing machinery, *sugarbabe* and *dawdle*, are transcriptionally targeted by REPTOR in the fat bodies. These findings suggest that *REPTOR* may be involved in molecular sensing of carbohydrate and functions in conversion from dietary carbohydrate to fat storage.

It has been known that REPTOR activity is regulated by mTOR complex 1 via phosphorylation. Our CRISPR-mediated screening in S2 cells has identified several kinases that regulate REPTOR activity. We further examined their effect on REPTOR activity by knocking down or overexpressing these candidates in the fat bodies. Immunostaining with anti-REPTOR antibody revealed that REPTOR nuclear localization increased when *Sgg* (the Drosophila homolog of glycogen synthase kinase 3) and *Cdk1*(Cyclin-dependent kinase 1) were overexpressed, suggesting that *Sgg* and *Cdk1* may play a positive role in regulating *REPTOR* transcriptional activity.

Together, these findings underscore the critical role of REPTOR in carbohydrate-lipid homeostasis within *Drosophila* fat bodies. We also identified novel regulators of REPTOR. Whether these kinases regulate REPTOR activity in the fat bodies under nutrient stress such as high sugar treatment is to be investigated.

797S **Identifying Roles for Chromatin Regulators in Stochastic Gene Expression During *Drosophila* Eye Development** Marina L Curchitser, Katalina N Li, Alison Ordway, Robert J Johnston Department of Biology, Johns Hopkins University

Cells with identical genomes take on distinct fates due to specialized patterns of gene expression. While developmental mechanisms often lead to highly reproducible cell fate decisions, in some cases, gene expression is stochastic, leading to random patterns of cell types that are unique to each individual. The mechanisms controlling stochastic fate decisions are not well understood. Within the Drosophila eye, two R7 photoreceptor subtypes are characterized by the expression of either Rhodopsin 3 (Rh3) or Rhodopsin 4 (Rh4). These R7 subtypes are distributed in a random pattern across the retina. Despite this random spatial pattern, Rh4 is consistently expressed in 67% of R7s in wild type flies. The specification of these two R7 subtypes is controlled by the transcription factor Spineless (Ss). When Ss is present, Rh4 is expressed, and in its absence, Rh3 is expressed. *ss* expression is controlled by the dynamic regulation of transcription and chromatin during development. An early-acting enhancer opens chromatin at the *ss* locus in precursor cells. In differentiating cells, the *ss* locus stochastically remains open or recompacts. In terminally differentiating R7s in which the *ss* locus is open, a late-acting enhancer reactivates *ss* expression. In contrast, in cells where the *ss*locus is closed, *ss* remains repressed. Here, we are conducting an RNAi screen to identify chromatin modifiers that regulate the chromatin state at the *ss* locus during development. One main focus of the screen is the Polycomb Group (PcG) factors, which repress gene expression. Calypso, a member of the Polycomb Repressive Deubiquitinase Complex, is a promising candidate to regulate the *ss* locus. After identifying additional candidates that regulate the stochastic ON/OFF decision, we will use DNA and RNA FISH to characterize how these proteins function throughout development to regulate *ss* expression. Gaining insight into these mechanisms will contribute to a more complete understanding of how cell fate decisions are regulated during development.

798S **Study of Dual-Function Transcription Factor Runt in *Drosophila melanogaster* Early Embryos** Isaryhia M Rodriguez, Angelike Stathopoulos BBE, California Institute of Technology

Runt (Run) is the founding member of the highly conserved Runt-related (Runx) family of DNA-binding transcription factors which act to activate and repress gene expression in dynamic spatial and temporal ways. In vertebrates, Runx plays a wide variety of roles in development where it is a critical factor for hematopoietic stem cell maturation, osteoblast differentiation, and the specification of pain-transmitting neurons. In *Drosophila melanogaster*, Run is a primary pair-rule gene that regulates other pair-rule genes and segment polarity genes by acting as both activator and repressor. The characterization of Run's functions in regulating two enhancers that support the expression of *sloppy-paired 1* (*slp1*) in *Drosophila* embryos supports this view. Previous studies by the Gergen lab have shown that *slp1* is a segmentation gene that is simultaneously activated and repressed by Run across unique domains of the embryo based on unique combinations of transcription factor (TF) co-occupancy at two critical enhancers. The ability of Run to simultaneously support activation and repression at one time-point, in distinct cells of the embryo, is ideal for perturbing Run globally and capturing unique effects across space. Specifically, by leveraging Run site-specific mutants we can study how impairing certain domains of the Run protein contributes to both activation and repression simultaneously in a study of *slp1*. One critical aim for this work is to leverage a set of domain-specific transgenic run mutant fly stocks, to further dissect the roles of specific Run TF domains in the regulation of Run target genes - *slp1* as well as other direct and indirect targets identified through genomic analyses. Specifically, we will assay phenotypes associated with *run* mutants predicted to relate to dedicated-repressor or dedicated-activator mutant proteins. We hypothesize that Run switches activity in a context-dependent manner based on co-occupancy of enhancers by other transcription factors. Furthermore, we propose that Run's highly conserved protein domains play distinct roles in modulating this transcriptional regulation in the context of targets such as *slp1*. The overarching goal of our work is to utilize a combination of genetic manipulation, genomics, and in-vivo study to approach characterizing a spatial and temporally switchable role for Run in early *Drosophila* embryonic development.

799S **Cell Reintegration in the *Drosophila* Follicular Epithelium: Exploring Non-Neural Roles of Neuron Development Transcription Factors** Evan B Ost[1], Tara M Finegan[2], Dan T Bergstralh[2] [1]Department of Biological Sciences, University of Missouri, [2]University of Missouri

Epithelia are conserved animal tissues formed of layers of cells that line body compartments, acting as protective and impermeable barriers. As monolayered (simple) epithelial tissues develop, mitotic daughter cells can be born outside of the tissue layer. Our lab previously described a mechanism called cell reintegration, where these misplaced cells are reincorporated into the tissue plane to preserve epithelial integrity. Reintegration relies on members of the Ig-superfamily Cell Adhesion Molecule (IgCAM) family, proteins that are best understood for their roles in axon guidance and at the neuromuscular junction (NMJ). In the *Drosophila* follicular epithelium, which surrounds developing oocytes and associated germline cells, the IgCAMs important for cell reintegration include Neuroglian, Fasciclin II, and Fasciclin III. These proteins show a rapid decrease in expression about midway through oogenesis, immediately after the follicle epithelium ceases to be proliferative. We therefore hypothesized we could identify additional factors in reintegration by surveying expression levels. Using single cell RNA-Seq data from proliferative and immediately post-proliferative follicle cells, we identified additional neuron development factors with similar expression patterns to these IgCAMs. Notable among these differentially expressed factors are Fruitless, Doublesex, Eyes absent, and Abrupt, known regulators of transcription in the nervous system. This suggests potential non-neural functions for these factors in the ovary. We are currently exploring the role of these 'neural' factors in the development of the *Drosophila* ovary through genetic manipulation and advanced microscopic imaging techniques.

800S **How temperature affects gene expression to maintain phenotypic robustness?** Genoveva Guerrero Jiménez, Fernando Casares Fernandez Gene regulation and morphogenesis, CABD

The life cycle of Drosophila melanogaster responds to temperature by being shorter at higher temperatures. Despite the variability in life cycle duration, many of its organs, such as the eyes, develop to nearly the same size within the viability limit. . However, there may be some changes if the duration is altered. How does the system adapt to temperature changes to maintain a robust phenotype? How does temperature affect gene expression and the functioning of gene regulatory networks? To try to understand these fundamental questions, we are generating single-cell RNA-seq datasets from D. melanogaster third instar larval eye discs at different temperatures (18 ºC, 25 ºC and 29 ºC). We would like to analyze whether, apart from differential gene expression between conditions, there are genes that show different levels of transcriptional variability, also called noise, in response to temperature, which could be responsible for the robustness of the developmental program and the phenotype. While obtaining sequencing results, we worked with previously published scRNAseq data from D. melanogaster third instar larval eye discs to study transcriptional variability. We found that there are different categories of genes depending on how variable they are. These categories show enrichment on Gene Ontology terms, as genes associated with the developmental trajectory from progenitor to differentiated photoreceptors are the most heterogeneous. Our analysis also shows that the average transcriptional noise for all genes is not homogeneous across all cells of the disc, with some cell populations having higher average noise than others.

801S **Temporal Control of Neurogenesis in Drosophila Development** Yunchong Zhao, Salvador Salazar, Zoie Andre, Michael Perry UC San Diego

Animal development requires precise spatial and temporal cues to direct differentiation, and this process must be robust to ensure proper development in varying environments and genetic backgrounds. While many years of research have focused on how precise spatial patterns of gene expression are established and used to direct cell fate specification, much less is known about the mechanisms that control developmental timing. Precise timing and communication are perhaps especially important during neurogenesis for the proper connection of axons and dendrites. During late L3 *Drosophila* development, as the retina is specified from posterior to anterior, newly differentiated photoreceptor axons extend into the brain to connect to newly born medulla neurons and must match with the correct partners with precise timing to build functional neural circuits. Within the developing medulla, neuroblasts (NBs), or neural stem cells, express a cascade of temporal transcription factors (tTFs) sequentially as they age and give rise to ganglion mother cells during each temporal expression window, which then give rise to neurons of different fates. This is hypothesized to be the main mechanism that generates the extensive neural diversity of the medulla. The tTF progression is cell-intrinsic and each tTF is necessary for the progression of the cascade.

We aim to investigate the role of transcription in the temporal regulation of NB progression. Focusing on one of the tTFs, Dichaete (D), we used CRISPR HDR to replace the endogenous core promoter of D with the one from Thisbe (Ths), which has a weak and non-paused promoter (characterized in previous work). This 150bp modification centered on the +1bp of a D-GFP protein-fluorophore fusion line. The modified line is homozygous viable and quantification shows that Ths-D-GFP intensity is significantly lower than WT D-GFP, indicating lower D protein expression in NBs. We then asked whether this change in how the gene is transcribed and the protein levels produced effect D temporal window duration. We quantified the proportion of D-expressing NBs and found that Ths-D-GFP NBs are present at significantly lower proportions than in WT controls. This suggests that the promoter swap line has a shorter D-expression temporal window. Future work will examine the duration of the temporal window directly using D-GFP live imaging and examine whether the shorter temporal window influences the number or types of neurons produced. Together, our data suggest that the rate at which D is transcribed could control temporal window duration.

802S **Computational analysis of nuclear organization using RD-SPRITE to identify sex-specific three-dimensional DNA/RNA contacts** Megan Carlson[1], Mukulika Ray[2], Erica Larschan[2] [1]Center for Computational Molecular Biology, Brown University, [2]Molecular Biology, Cell Biology and Biochemistry, Brown University

The 3D nuclear organization plays a crucial role in cellular functions via gene regulation. While many techniques like HiC, microC, and HiChIP have revealed the importance of 3D contacts in transcription, our understanding of how nuclear organization shapes transcript processing, including RNA splicing, is still limited. RNA splicing is vital for cell and sex-specific transcriptomes and is often disrupted in many human diseases. Therefore, research focusing on understanding how spatial organization regulates mRNA splicing in various contexts is integral to understanding health's genetic basis. Sex-specific splicing is conserved across different species, and many human neurological disorders are associated with splicing defects. Interestingly, many of these neurological disorders show gender biases. Thus, understanding how sex-specific splicing is regulated can improve management and therapeutics for these diseases.

To explore this question, we apply a novel technique, RNA-DNA Split-Pool Recognition of Interactions by Tag Extension, or RD-SPRITE, developed by the Guttman Lab at Caltech. While this technique has been previously applied in mammalian contexts, we have successfully used the method on Drosophila samples to examine global three-dimensional DNA and RNA contacts. As a widely studied genetic model with extensive research in the field of sex-specific splicing, Drosophila RD-SPRITE datasets have the potential to elucidate the complex regulatory mechanisms that shape sex-specific transcript processing via nuclear organization. I have customized the SPRITE computational pipelines for application to novel Drosophila datasets, allowing for the detection of higher-order structures within the nucleus and comparison of 3D contacts between sexes via characterization of sex-specific clusters. Using this technique, I have identified DNA-DNA, RNA-RNA, and DNA-RNA clusters in both male and female fly cell lines. Differential clustering patterns between sexes reveal the role of three-dimensional chromatin organization on alternative splicing-based gene regulation. In combination with this structural information, I identified how chromatin organization is associated with sex-specific splicing by combining RD-SPRITE splicing cluster data with previously identified differentially spliced genes and transcription factor/RNA-binding data to generate a global understanding of the relationship between nuclear organization and gene regulation.

803S **Modelling the Complete Enhancer Landscape of the Adult Fruit Fly** Eren Can Eksi[1,2,3], Swann Floc'hlay[1,2,3], Valerie Christiaens[1,2,3], Gert Hulselmans[1,2,3], Lukas Mahieu[1,2,3], Stein Aerts[1,2,3] [1]Department of Human Genetics, KU Leuven, [2]VIB.AI Center for AI & Computational Biology, VIB, [3]VIB-KU Leuven Center for Brain & Disease Research, VIB

With the Fly Cell Atlas, we obtained an atlas of gene expression profiles of more than 250 annotated cell types across the entire adult fly. Here, we set out to model the gene regulatory programs and the full landscape of genomic enhancers that underly these transcriptomes. For this, we first generated a scATAC-seq atlas of the adult fly using a combination of 10x scATAC-seq, HyDrop-ATAC and 10x sc-multiome. After quality control and filtering, our atlas contains 700,00 cells and 140,000 genomic intervals that are accessible in at least one cell cluster. We clustered cells by chromatin accessibility using topic modelling and transferred cell type annotations from the scRNA-seq atlas using multiome bridging and manual annotation. This transfer was performed at two hierarchical levels and at higher resolution, yielding more than 150 annotated cell types with matched transcriptome and chromatin accessibility profile. Next, we trained sequence-based deep learning models on the established atlas, called DeepFly. These convolutional neural networks take enhancer sequences as input and predict cell type specific chromatin accessibility. Next, we used AI explainability methods to extract important sequence features for cell type specific enhancers. These features include combinations and strength of transcription factor binding sites, their location, orientation and cooperativity preferences. Furthermore, we are utilizing the models to understand emergent gene regulatory features and enhancer syntax in the context of a whole adult animal which is possible due to the near complete landscape of cell types and regulatory genome DeepFly models are trained on. We are currently in the process of validating these models and our findings using in vivo reporter assays. The integrated scATAC-seq atlas of the adult fly and the accompanying gene regulatory models and deep learning models will represent a unique resource in explaining how the genomic regulatory code of a whole organism translates into cell types.

804S **Investigating the role of Rusty in Tansferrin-mediated iron delivery** Hila Maleki[1], Sattar Soltani[2], Song Wang[2], Kirst King-Jones[1] [1]Biological Sciences, University of Alberta, [2]University of Alberta

Iron homeostasis is crucial for cellular and systemic functions, requiring precise coordination of iron uptake, transport, and storage to avoid toxicity from excess free iron. In Drosophila melanogaster, Transferrin 1 (Tsf1), an iron-binding protein secreted from the fat body, plays a central role in systemic iron transport and is actively taken up by the prothoracic gland (PG), an endocrine iron-rich tissue essential for ecdysteroid hormone production. However, the mechanisms mediating Tsf1 uptake and trafficking remain incompletely understood.

Co-culture experiments with tissues (PG and fat bodies) isolated from Evi5-RNAi and Tsf1-GFP larvae demonstrated increased intracellular accumulation of Tsf1-GFP in the PG, suggesting that Evi5, a Rab-GTPase-activating protein, facilitates Tsf1 trafficking by regulating endosome recycling. Mass spectrometry data revealed that Evi5 interacts with a candidate transferrin-binding protein (which we named Rusty) that displays sequence homology to human heat shock proteins. Rusty appears to localize to the endomembrane system, suggesting that it may mediate Tsf1 capture and intracellular transport. Notably, Rusty also interacted with other proteins involved in the uptake, storage, and transport of iron, underscoring its potential role as a key component of the iron regulatory network. Interfering with Rusty function in the prothoracic gland caused moderate accumulation of heme precursors, consistent with the idea that iron delivery to the mitochondria was disrupted.

We are currently examining the subcellular localization of Rusty to establish whether i) Rusty and Tsf1 co-localize to endosomes, and to ii) identify other protein involved in Tsf1 uptake.

By characterizing Tsf1-mediated iron transport and its associated pathways, this work seeks to uncover conserved mechanisms critical for development and iron regulation. These insights may have implications for understanding human iron-related diseases, and inform therapeutic strategies targeting iron homeostasis.

805S **Determining the localization and function of centromere-derived transcripts in *Drosophila melanogaster*** Maddy O›Connor, Barbara Mellone Molecular and Cell Biology, University of Connecticut

The accurate segregation of chromosomes relies on the proper functioning of the centromere, a specialized region where the kinetochore and microtubules attach during cell division. The centromere consists of a unique chromatin domain containing H3 variant CENP-A, which recruits other essential centromeric proteins. If the centromere complex is not assembled properly, it can cause detrimental chromosome segregation errors, leading to cell death or aneuploidy. The mechanism behind how the essential *Drosophila* centromeric proteins (CENP-A, CENP-C, and CAL1) are recruited to the centromere has yet to be understood. Studies in human cells suggest that centromere-derived transcripts associated with centromeric proteins play a role in the recruitment of CENP-A and are then incorporated into the centromere complex. This has yet to be observed in *Drosophila melanogaster,* and it is still unknown how common this connection is across species due to the differences in centromeric sequences. Previous work revealed the composition of the centromeres of *D. melanogaster* and discovered that a centromere-enriched retroelement, *Jockey-3,* is transcribed and that the nascent RNAs remain associated with the centromeres they originate from. However, the significance of this localization and its role remains unknown. To determine if other centromere-derived transcripts are expressed and localize to the centromeres they originate from, I am in the process of analyzing mitotic chromosomes from larval brains with RNA FISH (Fluorescence In-Situ Hybridization) using probes designed for CENP-A associated simple satellite repeats. Once the localization of these transcripts is identified, I will determine if they are retained at the centromere through interactions with different centromeric proteins using CLIP (Cross-linking Immunoprecipitation). This assay uses UV irradiation to irreversibly crosslink RNA to protein, followed by immunoprecipitation and RNA-seq to identify protein-bound transcripts. To determine if centromere-derived transcripts contribute to centromere integrity, I will use a CRISPR-mediated RNA degradation approach to remove them and identify any effects on the centromere complex. The findings from this work will enhance our understanding of the role transcription plays in centromere function and its impact on chromosome segregation across various species.

806T **Role for Moesin in the germline of the developing egg chamber** Raegan Mozal, Allyson Foster, Izabella Jordan, Lindsay Lewellyn Butler University

Defects in egg development, or oogenesis, can lead to infertility; therefore, studying the structures and pathways necessary for successful oogenesis could lead to better diagnosis and treatment of this condition. Intercellular bridges are essential structures found in the developing eggs of many animals. The fruit fly egg chamber provides a powerful model system to study the formation and growth of intercellular bridges. Each fly egg develops from an egg chamber composed of one oocyte and fifteen nurse cells surrounded by a layer of somatic epithelial cells. The sixteen germline cells are connected by intercellular bridges, or ring canals, which increase in diameter to allow material to be transported from the nurse cells into the oocyte. Ring canal stability and growth require coordination between the nurse cell membrane and the underlying actin cytoskeleton. The ERM protein, Moesin, is known to link membrane proteins to the actin cytoskeleton, and the active, phosphorylated form of Moesin has been shown to localize to the nurse cell membranes and around the germline ring canals. Therefore, we hypothesize that Moesin could stabilize the germline ring canals and/or promote their growth. To test this hypothesis, we have used the dominant female sterile technique to generate homozygous mutant germline clones for each of three different *moe* mutations, and we have monitored ring canal size as well as the size of the mature eggs that develop from these egg chambers. In addition, we have tested whether germline expression of a phosphomutant (T559A) or phosphomimetic (T559D) form of Moesin in the germline affects ring canal size or stability. In the future, we hope to determine how Moesin activity is regulated in the germline.

807T **Specialized translational machinery is required for spermatogenesis in *Drosophila melanogaster*** Brook L Falk[1,2], Yonit Tsatskis[2], Julie A Brill[1,2] [1]Molecular Genetics, University of Toronto, [2]Cell Biology, Hospital for Sick Children

Drosophila melanogaster sperm development relies on extensive post-transcriptional regulation as thousands of transcripts are preserved to be translated at later stages when they are required. A key step in translation initiation is binding of eukaryotic initiation factor 4E (eIF4E) to the 5' mRNA cap. *D. melanogaster* has multiple paralogues of *eIF4E*, including four testis-enriched paralogues (*eIF4E3*, *4*, *5* and *7*). We and our collaborators previously discovered that eIF4E3 and 5 are both needed for male fertility and formation of mature sperm. Their roles, however, are stage specific as eIF4E3 is required for meiotic chromosome segregation and cytokinesis whereas eIF4E5 is required for the final stage of spermiogenesis called individualization. During individualization, organelles and cytoplasmic components not needed in mature sperm are stripped away by non-apoptotic caspase activity. In flies lacking eIF4E5, caspase activity is dysregulated, resulting in the absence of mature sperm. Thus, we hypothesize that eIF4E5 is a stage specific translational regulator required to ensure correct spatiotemporal synthesis of proteins involved in individualization. Members of the eIF4E family have structurally conserved C-terminal regions that interact with the mRNA cap and non-conserved, disordered N-termini. Thus, the specific functions of the testis eIF4Es are likely conferred by differences in gene expression, mRNA localization and translation, or protein sequence. We generated transgenes to define the gene and amino acid sequences that are required for eIF4E5 function and found that expression the conserved C-terminal mRNA binding region of the eIF4E5 open reading frame under the control of a spermatocyte specific promoter (*b2-tubulin)* is sufficient to restore fertility in *eIF4E5* mutants. To further the mechanistic understanding of eIF4E5, I generated functional eIF4E5 transgenes tagged with promiscuous biotin ligase miniTurbo to identify transient protein interactors. I have also found that eIF4E5 is concentrated at the tail ends of individualizing sperm cells. Interestingly, a group mRNAs called "cups" and "comets" are known to localize to this subcellular region, suggesting that they may be regulated by eIF4E5 in a location dependant manner. This hypothesis will be tested using RNA-immunoprecipitation and sequencing to identify mRNAs that coelute with eIF4E5. Ultimately, our results will provide insight into how eIF4E5 functions and is regulated during spermatogenesis.

808T **Female germ cell identity depends on an X-linked H3K9me3 mini-silencing domain** Helen Salz Genetics and Genome Sciences, Case Western Reserve Univ

Germ cell development is sexually dimorphic, culminating in the production of either sperm or eggs. In Drosophila, when female identity is compromised, germ cells cannot differentiate and instead form tumors that ectopically express hundreds of spermatogenesis genes. Remarkably, dysregulation of one downstream spermatogenesis gene, *PHD finger protein 7 (phf7),* is sufficient to activate a sex-inappropriate gene expression program and drive tumor formation. Thus, sex-specific control of *phf7* is a critical regulatory checkpoint for the male/female fate pathway. We recently discovered that *phf7* is silenced by a gene-specific mini-domain of trimethylation of histone H3 lysine 9 (H3K9me3) marked chromatin in female germ cells. Of the three enzymes known to methylate H3K9, only SETDB1 plays a role in silencing *phf7*. SETDB1 is also required for TE silencing, where a piRNA-guided mechanism recruits SETDB1 and instructs local H3K9me3 deposition. However, we found that *phf7* is silenced by a piRNA-independent mechanism. We report here that silencing requires two DNA binding proteins: Stonewall (STWL), a member of the MADF-BESS protein family, and Identity Crisis (IDC), a member of the ZAD-ZNF protein family. Loss of either *idc* or *stwl* prevents H3K9me3 deposition and *phf7* silencing. IDC and STWL localize to adjacent regulatory elements within the *phf7* gene, but IDC only associates with *phf7* in female germ cells. Together, these studies demonstrate that while both IDC and STWL are necessary to build the H3K9me3 silencing domain, IDC localization provides female-specificity. Although H3K9me3-mediated silencing is a conserved strategy for repressing developmentally regulated protein-coding genes, the mechanism controlling lineage-specific installation of this epigenetic mark is poorly understood. By focusing on a single biologically relevant gene, our studies may reveal a general mechanism for context-dependent establishment of H3K9me3 silencing domains.

809T **Characterization of the role of Phosducin-Like Protein 3 in gametogenesis** Jennifer C Mierisch, Gabi C Rant, Anthony C Roukoz, Grace C Flemming, Christopher C Petit, Samantha C Webster, Stefan C Kanzok Biology, Loyola University Chicago

Approximately 17% of couples worldwide experience infertility with a prominent cause being a failure to produce quality sperm and egg. To understand the underlying causes, it is necessary to identify the molecular mechanisms regulating gametogenesis. Recent studies in the lab have identified Phosducin-like protein 3 (PhLP3), the *Drosophila* homologue of human TXNDC9, as a key regulator of gametogenesis. PhLP3 is expressed in the testis and ovary and belongs to the PhLP3 family of proteins, which contain a conserved thioredoxin domain and have been shown to function as co-chaperones in the folding of cytoskeletal proteins. *PhLP3* mutant males and females exhibit sterility and reduced fertility, respectively, leading us to explore how PhLP3 functions in gametogenesis. Analysis of male *PhLP3* mutants reveals defects in the late stages of sperm maturation, including scattered spermatids, abnormal nuclear elongation, and failed individualization, resulting in seminal vesicles devoid of mature sperm. Using transmission electron microscopy to further examine these mutants at a higher resolution, we observe significant cyst disorganization and defect in mitochondrial derivative morphology. Interestingly, flagella still elongate and axonemes exhibit the characteristic 9+2 arrangement of microtubules. To determine where the PhLP3 protein localizes and if the conserved thioredoxin activity is critical for its function in spermatogenesis, we have utilized CRISPR to tag the protein and to mutate its thioredoxin activity. Studies are underway to explore the subcellular localization of PhLP3 and the effects of thioredoxin domain mutation. Female PhLP3 mutants also exhibit reduced fertility, producing eggs that largely fail to hatch. Immunostaining suggests that PhLP3 is expressed in the somatic follicle cells of the developing egg chamber in the ovary, which are known to regulate egg shape via interactions with the underlying basement membrane. We are currently examining the effects of *PhLP3* mutation on egg development, and the localization of PhLP3 with components of the cytoskeleton in the egg. The high level of conservation of PhLP3 and its homologs suggest that our findings regarding the role of PhLP3 in gametogenesis will be broadly applicable across species.

810T **Identifying Meiotic Proteome via TurboID-based Proximity Labeling** Oscar B Bautista[1], Janvi Ramachandran[1], Zion Tasew[2], Ella McLaren[2], Nicole Crown[1] [1]Biology, Case Western Reserve University, [2]Case Western Reserve University

The preservation of genome integrity hinges upon the precise segregation of replicated chromosomes throughout eukaryotic mitotic and meiotic cellular divisions. Failure to properly segregate chromosomes, facilitate homologous recombination, or repair DNA damage in meiosis in mitosis are main causes of infertility and cancer. Meiosis is initiated by programmed DNA double-stranded DNA breaks (DBSs) which are repaired as crossovers (CO), reciprocal exchanges of genetic information between homologous chromosomes. To mediate structural integrity and proper pairing of homologous chromosomes, a tripartite structure known as the synaptonemal complex (SC) forms along the entire length of chromosomes. Meiotic proteins that carry out key processes (DSB formation, SC formation, and CO formation) have undergone significant molecular evolution across taxa, which has increased the difficulty to identify meiotic genes. Efforts over the past 50 years have identified meiotic proteins with much success. However, mutagenesis screens have not yet identified crucial meiotic proteins homologs (such as MutLy and the ZZS complexes). To address these gaps, we are utilizing proximity labeling to expand on the list of meiotic proteins in *Drosophila*. We have generated fly lines that contain an engineered biotin ligase TurboID conjugated to Vilya and Mei-218, enabling them to biotinylate proteins in a 10 nm radius. Utilizing these lines, we have confirmed through confocal microscopy that these endogenously tagged TurboID proteins biotinylate proteins along the synaptonemal complex, the zipper-like protein structure that forms during meiosis to keep homologous chromosomes together. Additionally, we have shown that we can isolate these biotinylated proteins with magnetic streptavidin beads and identify the biotinylated proteins through mass spectrometry. Currently, we are conducting RNAi screening on high-confidence hits to identify proteins that are implicated in meiosis. The protein-protein interactions uncovered will also provide insight into the complex regulatory mechanisms that are required to form COs.

811T **Investigating the role of female-derived sperm-associated proteins in fertility and reproduction** Melissa Mychalczuk[1], Enisa Aruci[2], Mehrnaz Afkhami[1], Mariana Wolfner[1] [1]Molecular Biology and Genetics, Cornell University, [2]Centre des Sciences du Goût et de l'Alimentation

During mating, sperm are transferred from a male to a female, where they complete functional maturation in the female reproductive tract (FRT) in order to fertilize eggs. In the FRT of Drosophila melanogaster, both male and female-derived proteins associate with sperm, as characterized by McCullough et al. 2022 using mass spectrometry and sex-specific isotope labeling. While the functions of several male proteins have been characterized, less is known about interactions between female proteins and sperm. Many such proteins have canonical roles (metabolic enzymes, transcription and translation machinery, etc.) while others are uncharacterized, but few have been investigated in the context of reproduction. We are using genetic tools (CRISPR/RNAi) to identify sperm-associated female proteins that are important for sperm function and reproductive success, beginning with metabolic enzymes identified by McCullough et al. We assay fertility, hatchability, and sperm storage to identify defects in reproduction upon depletion of each protein. These findings, along with information on the localization of these proteins in or on sperm, will provide novel insights into the female-mediated mechanisms of sperm maintenance and storage in the time preceding fertilization.

812T **Uncovering the mechanisms of sterility caused by *me31B* gene mutations through multi-omics profiling** Ming Gao[1], Raheem Mansoor[1], Deep Govani[1], Brynn Nylin[1], Evan Kara[1], Jenna Ibrahim[1], Abraham Fielder[1], Ana Iglendza[2], Jonathan Trinidad[3] [1]Indiana University Northwest, [2]Loyola University Chicago, [3]Indiana University

The *Drosophila me31B* gene, encoding an essential RNA helicase for female germline development, is critical to fertility. In previous work, we identified three sterility-causing alleles of *me31B*: $me31B^{E208A}$ (DEAD-box mutation, dominant sterility), $me31B^{C\text{-}ter}$ (deletion of N-ter domain, recessive sterility), and $me31B^{H333R}$ (QxxR motif mutation, recessive sterility). However, the precise molecular disruptions driving sterility in these mutants remain unclear. This study addresses this gap through a multi-omics approach. We analyzed ovaries from these mutants using total mRNA sequencing, whole-ovary proteomics, and Me31B-targeted IP mass spectrometry to characterize transcriptomic, proteomic, and interatomic changes associated with each allele. Our findings reveal key alterations in genetic and protein networks, providing novel insights into the mechanisms by which *me31B* domain and motif mutations compromise fertility. This research provides new leads and directions for further analysis of Me31B›s molecular-level role in germline development.

813T **The N-terminal domain and QAHR motif of Me31B are needed for *Drosophila* germ cell formation** Ming Gao[1], Evan Kara[2], Abraham Fielder[3], Adriana Iglendza[3] [1]Indiana University Northwest, [2]Marian University, [3]Loyola Univeristy

Me31B is an ATP-dependent DEAD-box RNA helicase abundantly found in *Drosophila* female germline. Together with other germline proteins and RNAs, Me31B forms Ribonucleoprotein (RNP) complexes that function to silence germline mRNA transcripts until they have localized to the correct location: a process critical for proper germ cell formation. Despite this role, the molecular mechanism of how Me31B contributes to germ cell formation needs to be better understood. To study this, we directed our attention to two *me31B* mutations recently reported to cause female sterility: $me31B^{C\text{-}ter}$ (N-terminal domain deletion) and $me31B^{H333R}$ (point mutation in the QAHR motif). To analyze germ cell formation in the mutants, we immunostained stage 10-11 embryos with anti-Vasa antibodies to mark the germ cells and quantified the germ cells. We observed a significant reduction in germ cell number in both mutants (32% lower in $me31B^{H333R}$ and 36% lower in $me31B^{C\text{-}ter}$). Then, to find out whether this reduction is caused by abnormalities of Osk protein (the protein that induces the formation of germ plasm: the special cytoplasm that directly leads to germ cell formation), we immunostained Oskar in both ovaries and early-stage embryos to check for phenotypes in Osk expression or localization. While no obvious phenotypes were observed during oogenesis, Osk in the early embryos showed a reduction in protein quantity and/or mis-localization in both mutants. These findings suggest the role of Me31B's N-terminal domain and QAHR motif in regulating Osk protein levels, localization, and germ cell formation.

814T **Characterizing the composition and morphology of the germ plasm in the wasp *Nasonia vitripennis*** Allie Kemph[1], Kabita Kharel[2], Samuel Tindell[2], Alexey Arkov[2], Jeremy Lynch[1] [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 surprising variability in morphology and composition of germ plasm particles among animals. 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 preceding pole cell formation. While much is known about the composition of the fly germ plasm, how the structure of the oosome compares is as yet unknown. Here we describe our progress in characterizing the dynamic oosome morphology and determining the spatial arrangement of several oosome mRNA and protein components. In contrast to the arrangement of these components in polar granules several highly enriched mRNAs seem to form a mesh-like network, Tudor protein is enriched at the periphery of the pre-blastoderm stage oosome and the examined mRNAs do not co-occupy internal Vasa/Oskar protein granules. This work has also revealed that mitochondria appear to be excluded from the oosome while ribosome-associated vesicles are ubiquitously distributed throughout.

815T **Identification of a Potential New Protein Required for Proper Drosophila Meiotic Double-Strand Break Formation** Bowen Man[1], Bowen Man[2] [1]Case Western Reserve University, [2]Biology, Case Western Reserve University

Recombination is essential for proper gametogenesis in sexually reproducing organisms, initiated by the formation of double-strand breaks (DSBs) through tightly regulated mechanisms in germline cells. In *Drosophila melanogaster*, six proteins—Mei-W68, Mei-P22, Trem, and three RING finger domain-containing proteins (Vilya, Narya, and Nenya)—have been identified as necessary for proper DSB formation in female germline cells. Here, we present evidence that a previously uncharacterized protein, CG14410, is a potential new member required for proper DSB formation in female *Drosophila* germline cells.

816T **The interplay of the gut microbiome and diet on oogenesis** Taylar J Mouton, Katilyn Harrison, Nichole Broderick Biology, Johns Hopkins University

Animal gut microbes impact several aspects of host physiology. In humans, these microbes, or the microbiome, contribute to a growing number of host disease states. However, the high complexity of the human gut microbiome makes it harder to isolate variables and define mechanisms. Additionally, it is nearly impossible to study how the previous generation's microbial status impacts their offspring and subsequent generations. Thus, model organisms, such as *Drosophila melanogaster* have provided a valuable tool to study host-gut-microbiome interactions. The *D. melanogaster* gut microbiome is relatively low-complex and these microbes can be readily cultured in the lab environment. Interestingly, some studies have reported the parental gut microbiome status can exert unexpected consequences on offspring development and physiology, spanning for multiple generations. While these studies indicate the gut microbiome impacts host physiology and development intergenerationally, they have attributed different bacterial strains and signaling pathways to these impacts. Therefore, it is still unknown how exactly the microbiome could be impacting early development to contribute to the faithful maintenance of processes like oogenesis. The goal of this research was to characterize the impact of the gut microbiome on *D. melanogaster* oogenesis. To this aim, female flies were reared with microbes or without microbes for three generations, aged five days and allowed to lay, and then sacrificed for ovary morphology analysis. We found that across generations, the number of mature eggs and number of stage ten follicles is significantly different between females reared with microbes compared to those reared without, whereas the number of dying follicles was not significantly different. Interestingly, the impact of microbes on these phenotypes was diet-dependent; these metrics were significantly different on a low yeast diet, but were not different on a high yeast diet. These results suggest that microbes have negative consequences on oogenesis over time when flies are reared on low yeast as compared to when reared on a higher yeast diet. Therefore, rearing conventional or microbe exposed flies on a higher yeast diet is more beneficial for faithful oogenesis over multiple generations. Altogether, our data highlight the role of the diet in influencing interactions between the host and its microbes, which can in turn have generational consequences.

817T **Investigating how germline sexual identity controls sex-specific gene expression** Harrison A Curnutte, Mark Van Doren Biology, Johns Hopkins University

In nature, animals often exhibit sexual dimorphism, or differences in morphology and behavior between sexes. An important difference between the sexes are the gonads, the testis and ovary, which produce the sperm and eggs necessary for sexual reproduction. To develop proper gametes, both the germ cells and the somatic cells of the gonad must decide their sexual identity. In many animals, the sex of the somatic gonad instructs the sexual identity of the germline. However, in animals like mammals and Drosophila, the sex chromosome constitution of the germ cells also impacts germline sexual identity. Thus, how signals from the soma combine with the germ cell's autonomous sexual identity to specify germline sex determination is an important question in the field. The female-specific RNA binding protein Sex lethal (Sxl) is expressed according to the presence of two X chromosomes and has been shown to be essential for both somatic and germline sexual identity. While we understand how Sxl regulates somatic sex, how Sxl acts in the germline is less well understood.

A key aspect of sex determination is the control of sex-specific gene expression, about which little is known in the germline. We have found that Tudor domain-containing protein 5-like (Tdrd5l) is expressed male-specifically in the undifferentiated germline and is important for male germline sexual identity. The mammalian homolog of *Tdrd5l*, *TDRD5,* is also required for spermatogenesis in mice, indicating that its role may be conserved throughout the animal kingdom. We have found that *Tdrd5l* is likely a direct target for post-transcriptional regulation by Sxl. Surprisingly, we have also found that *Tdrd5l* expression is regulated at the level of transcription. We have identified a small region of the *Tdrd5l* promoter that regulates both germline-specific and male-specific expression. We have also found that male-specific *Tdrd5l* transcription does not depend on *Sxl* alone, and is influenced by the sex of the surrounding soma. However, *Tdrd5l* is regulated independently of JAK/STAT signaling, which is the only known signal regulating sex-specific germline gene expression. Our study indicates that transcriptional regulation by a previously unidentified somatic signal in combination with sex chromosome constitution read out by Sxl regulates sex-specific germline gene expression of *Tdrd5l*.

818T **A novel network of BTB-DBD proteins controls sex-specific development of the *Drosophila* gonad** Samantha C Goetting[1], Lydia Grmai[1,2], Mark Van Doren[1] [1]Biology, Johns Hopkins University, [2]University of Pittsburgh

In the animal kingdom, most species exhibit sexual dimorphism, or phenotypic differences between the sexes. In *Drosophila*, the key factor controlling sexual dimorphism is Doublesex (Dsx). Dsx is the founding member of the Doublesex/Mab-3 Related Transcription Factor (DMRT) family that controls sex-specific gonad formation in most or all animals, including humans. Our lab has shown that Dsx regulates steroid hormone Ecdysone (E) signaling through female-specific Ecdysone Receptor (EcR) expression. E functions to initiate ovary development, while the lack of E signaling is important for the testis to maintain its male fate. Bioinformatic analyses have been used to predict Dsx targets, and many are transcription factors that contain a DNA binding domain (DBD) along with a BTB (Broad-complex, Tramtrack, and Bric-a-brac) domain that promotes homo- and heteromeric BTB protein interactions. Here we focus on three of these factors: *broad (br), chinmo (chronically inappropriate morphogenesis)* and *mamo (maternal gene required for meiosis)*. These genes are critical for gonad development and are predicted to be regulated at the level of Dsx and E signaling.

We have found that *mamo* is essential for ovary development, and *mamo* loss leads to a female-specific phenotype with severely disorganized adult ovaries and loss of egg production. Conversely, *chinmo* is essential for male gonad identity. Consistent with this, Mamo is expressed in the female somatic gonad while Chinmo becomes restricted to males. Our data indicate that both time- and sex-specific regulation of these genes is controlled by Dsx and E signaling in the developing gonad. During the larval stage, female-specific E signaling activates Br, which both represses Chinmo and promotes Mamo to initiate ovary development. Further, either loss of *chinmo* or ectopic E signaling in males feminizes the testis and activates Mamo. Chinmo and Br are mutually inhibitory, ensuring that the correct developmental pathway is activated in each sex. Thus, we have identified a key network of BTB-DBD transcription factors that mediate how Dsx and sex-specific E signaling control gonad sexual dimorphism. Given that DMRTs and steroid hormones control sexual development in other animals including humans, characterizing genes critical for sexual identity is of great importance for reproductive health.

819T **The Fluorescent Ubiquitination-based Tribbles Turnover Indicator (FUTTI) reveals sites of Trbl activity in tissue** Shima Shayestehpour[1], Leonard Dobens[2] [1]BBS, UMKC, [2]BBS, Univ Missouri, Kansas City

The conserved pseudokinase Tribbles (Trbl) regulates cell growth, division and differentiation by binding and mediating the degradation of the key growth regulators, including Akt kinase, String-Cdc25 phosphatase and the transcription factor Slbo-C/EBP, responsible for cell migration events in the anterior follicle cells at late stages of oogenesis. Protein sequence alignment of Slbo with mammalian C/EBP family members revealed a conserved motif responsible for C/EBPa binding human to Trib1 in vitro. Here we show that mutagenesis of this degron motif in an HA-tagged Slbo transgene resulted in both increased HA accumulation in anterior follicle cells and ectopic HA expression in mainbody FC. These data indicate that low levels of Trbl activity are required for Slbo turnover in anterior cells and high levels of Trbl activity are necessary in posterior cells, specifically those that contact anterior cells, to maintain very low levels of Slbo. To examine better the complex signaling regulating Trbl activity in this and other tissues, we fused the Slbo degron motif to a ubiquitously-expressed GFP indicator to generate a Fluorescent Ubiquitination-based Tribbles Turnover Indicator (or FUTTI) and produced transgenic flies bearing this construct. Preliminary evidence shows that GFP-degron levels in FUTTI wing disks are sensitive to Trbl misexpression and we will present data using this tool to probe the tissue-specific regulation of Tribbles-Slbo interactions in the ovary and to dissect the conserved features of the Tribbles protein required for targeted protein degradation.

820T **The RNA-binding protein Syp mediates translational repression of a cohort of spermiogenesis transcripts by binding to their 5'UTRs** Catherine Baker, Neuza Matias, Cordelia Li, Margaret Fuller Stanford Univ Sch Medicine

In the male and female germline, the spatial and temporal regulation of protein expression often relies on control of RNA stability, localization, and translation. In the *Drosophila* testis, ~1800 genes are expressed for the first time in mid-stage spermatocytes, under the direction of the Aly/tMAC complex; another 1200 genes show expression from a novel, Aly/tMAC-dependent promoter. Most of the transcripts from the class of 1800 newly-expressed genes are important for post-meiotic differentiation and are not translated until 1-3 days after the meiotic divisions, suggesting that these RNAs are subject to translational repression in spermatocytes and early spermatids. A candidate for executing such a translational repression program on a broad swath of RNA targets is the RNA-binding protein Syp, the fly homolog of human SYNCRIP. In work published recently, we performed CLIP-Seq to show that a spermatocyte-specific version of Syp was bound to the *cycB* 3'UTR *in vivo*, where it acts to promote CycB protein expression in mature spermatocytes (Baker et al., 2023). Subsequent analysis of the CLIP-Seq data revealed that Syp was bound at high levels to the 5'UTRs of many transcripts that require Aly/tMAC for their expression, particularly those whose protein products do not appear until late in spermatid development. In addition, Syp binding was enriched at AAAUU motifs, consistent with the Syp consensus site identified *in vitro* by the Hughes lab (Ray et al., 2013). To test whether the presence of Syp at 5'UTRs could be mediating translational repression, we created eYFP reporter lines driven by the promoter and 5'UTR of transcripts bound by Syp and assayed eYFP expression live in spermatocytes and spermatids. The 5'UTRs for three genes (*ocn*, *Mst84B*, and *TSSK*) repressed translation of eYFP up until late spermatid stages in a wild-type background but not in a *syp* mutant. In fact, all three reporters were expressed robustly in spermatocytes and early elongating spermatids when Syp was absent. Notably, all three of these 5'UTRs contain at least two AAAUU motifs, over which Syp CLIP signal is centered. In contrast, the 5'UTR of *Theg* shows Syp bound to only one AAAUU, and this 5'UTR was not sufficient to repress eYFP translation in wild type spermatocytes. Curiously, Syp binds the 5'UTRs emanating from each of its two spermatocyte-specific promoters, suggesting that it may repress its own translation after an initial burst of Syp protein production early in spermatocyte development. We will test this possibility with each *syp* spermatocyte promoter and 5›UTR driving destabilized GFP in wild type and the *syp* mutant, to better detect Syp-dependent translational repression of the reporters shortly after they are first expressed. Expression levels of the HA-Syp protein decrease after the meiotic divisions and become undetectable in late spermatids, likely allowing Syp target RNAs to get translated.

821T **Revisiting Male Infertility Caused by X-Autosome Translocations: New Insights from Cytogenetic Analyses in Drosophila** Camila Avelino[1,2], Mara MLS Pinheiro[1], Kayla Ho[3], Samantha Daly[3], Timothy Karr[2], Maria Vibranovski[1,3] [1]Genetics and Evolutionary Biology, University of São Paulo, [2]Biodesign Institute, [3]New College of Interdisciplinary Arts and Sciences

In 1972, Lifschytz and Lindsley (1) reported in a classic study that X-autosome translocations in *Drosophila melanogaster* led to male infertility in 75% of cases, likely due to disruptions in Meiotic Sex Chromosome Inactivation (MSCI). In contrast, translocations involving only autosomes rarely cause male infertility, highlighting the unique regulatory demands of the X chromosome during spermatogenesis. During MSCI, the X chromosome is confined to a specific nuclear territory where active RNA polymerase II (RNA Pol II) is typically absent, thereby ensuring the transcriptional silencing that is crucial for proper sperm development (2).

Our study revisits this issue by investigating how chromosomal positioning within nuclear territories influences MSCI and fertility. Using fluorescent in situ hybridization (FISH) with oligopaints and immunofluorescence, we analyzed *Drosophila* strains with X-2nd chromosome translocations. In sterile males, reciprocal translocations misplace the X chromosome euchromatin into an autosomal territory, where MSCI fails. This is evidenced by persistent RNA Pol II Serine 2 phosphorylation on the X chromosome, which would normally be suppressed to ensure silencing.

Conversely, in fertile males with non-reciprocal translocations, the integration of the 2nd chromosome's euchromatic region into the X territory does not disrupt MSCI. Despite part of the 2nd chromosome occupying the X domain, it remains transcriptionally active due to RNA Pol II staining, while the X chromosome retains its silencing.

Our findings suggest that nuclear organization plays a fundamental role in MSCI. It appears that only translocations repositioning the X chromosome into an autosomal territory may lead to infertility, likely due to altered transcriptional control. These observations further highlight the potential importance of chromosomal spermatocyte territories in preserving male fertility.

(1) Lifschytz E and Lindsley DL. The role of X-chromosome inactivation during spermatogenesis. Proc Natl Acad Sci USA. 1972;69:182-6

(2) Mahadevaraju S. et al. Dynamic sex chromosome expression in *Drosophila* male germ cells. Nat Commun. 2021;12:892.

822T **Utilizing Single-Cell Mass Spectrometry Methods to Quantitatively Profile Egg Development in *Drosophila Melanogaster*** Merin M Rixen, Rachel M Ogorzalek Loo, Joe A Loo, Margot E Quinlan Chemistry and Biochemistry, UCLA

In the United States, about 9% of men and 11% of women face fertility issues, with women experiencing a greater decline in fertility as they age. My work focuses on studying egg development, or oogenesis, an evolutionarily conserved process that produces mature ova ready for fertilization. I am leveraging the vast tools of the genetically tractable model organism, *Drosophila melanogaster* (the fruit fly), which is widely used to study oogenesis. The Drosophila ovary contains egg chambers that progress asynchronously through 14 morphologically distinct stages, undergoing dynamic changes in protein expression. A powerful way to determine function is to examine these protein expression patterns. Transcriptomics data revealed that most mRNA levels do not change throughout oogenesis, demonstrating a need for proteomic analysis. Although mass spectrometry has been used to analyze various stages of the Drosophila life cycle, protein expression in the whole ovary has yet to be explored. While a few groups have studied the proteomes of mature eggs, there is a lack of data covering the full range of developmental stages of oogenesis. A key challenge is isolating egg chambers from specific stages in sufficient quantities for comparative analysis. To overcome this challenge, I will employ single-egg-chamber mass spectrometry, enabling precise data collection from specific stages of oogenesis. Using this method, I will collect time-resolved proteomic data throughout oogenesis, selecting proteins that are differentially expressed for further study. I expect differential protein expression patterns between stages of oocyte development to provide invaluable information about oogenesis and fertility. To date, we have obtained data for stages 9 and 11 of egg development, identifying 677 proteins in stage 9 and 1920 proteins in stage 11. Proteomic analysis will be supported by the extensive datasets and genetic resources available for Drosophila research. I will use RNAi and overexpression to test candidates identified in our proteomics screen for a role in fertility. Additionally, I will compare the ovarian proteome to other stages of the Drosophila life cycle, integrating transcriptomics and smFISH. By identifying structural and regulatory elements enriched during distinct developmental stages, I aim to uncover molecular pathways associated with infertility, which can be further studied for their impact on fertility and potential as treatment targets.

823T **Characterization of testis-specific sugar transport and glycolysis genes in *Drosophila melanogaster*** Mark Hiller Goucher College

During spermatogenesis, germline cells develop into sperm while completely surrounded by two somatic cyst cells. How sugars molecules pass across the cyst cell barrier and enter the germline cells is not understood. Twenty-five genes that are annotated to encode SLC2 type sugar transporters are in the *Drosophila melanogaster* genome, and five of the sugar transporters appear to be expressed only in the testis. Mutations in each of three predicted transporters, *sut3*, *sut4*, or *CG14605*, are fertile. An alternative model is that pyruvate generated in the cyst cells is converted to lactate that is then transported from cyst cells to the germline cells. The enzyme lactate dehydrogenase (LDH) converts pyruvate to lactate, which could be converted back to pyruvate in germline cells and used to generate ATP. In addition to LDH, the *Drosophila* germline encodes one potential testis specific homolog of lactate dehydrogenase. Likewise, there are several genes that function in glycolysis that have testis specific homologs or testis specific spice forms. To assess the role of glycolysis and lactate transport in germline and cyst cells, we are using RNAi to knockdown function of genes that potentially play a role in the lactate shuttle between cyst and germline cells.

824T **Primordial germ cell migration requires lipid-mediated autophagy** Marcus D Kilwein[1,2], Akira Nakamura[3], Stanislav Shvartsman[4], Elizabeth Gavis[5] [1]Princeton University, [2]QCBx, Simons Foundation, [3]Department of Germline Development, Kumamoto University, [4]Molecular Biology and LSI, Princeton University, [5]Molecular Biology, Princeton University

Here we report that autophagy is a unique requirement of primordial germ cells. Previous work has shown that bulk autophagy is not required for embryogenesis in Drosophila, with mutants for essential autophagic machinery surviving until late larval stages. However, proximity based proteomic analysis by our lab revealed accumulation of phosphatidylinositol(3,5) P2[PI(3,5)P2]-based autophagic machinery in the primordial germ cells. Autophagy associated proteins detected include the kinases PI3K59F and Fab1, which sequentially add phosphates to a PI backbone, generating first PI(3)P and then PI(3,5)P2, as well as several effector proteins including VPS35, Hrs, and ATG18a. To confirm the activity of the two kinases in the germline, we generated a Dualsensor which simultaneously detects PI(3)P and PI(3,5)P2. The Dualsensor reveals a 5-fold increase of PI(3,5)P2 in the germline relative to the soma at the onset of gastrulation. We then generated endogenously tagged Fab1, and found that Fab1's association with PI(3,5)P2 positive endosomes is specifically increased in the primordial germ cells, supporting increased activity. We find that the PI(3)P effector protein VPS35 accumulates on germline endosomes, generating the tubules characteristic of VPS35 activation. Additionally, ATG18a and V-ATPase, two known PI(3,5)P2 effectors, accumulate on germline endosomes. Finally, microinjection of a Fab1 inhibitor strongly impairs primordial germ cell migration to the gonad without affecting somatic development or hatching. Thus, our data show that primordial germ cells uniquely require autophagic machinery, and that autophagic machinery is facilitating primordial germ cell migration to the gonad.

825F **Investigating kinetochore – microtubule attachments and chromosome movement in *Drosophila* Meiosis** Madeline Terry[1], Shruthi Gunturu[1], Kim McKim[2] [1]Genetics, Rutgers University, [2]Rutgers University

During female meiotic cell division, the interactions between the kinetochores (KT) and the spindle ensure the correct partition of chromosomes. The establishment of end-on attachments of homologous KTs to opposite spindle poles is known as *biorientation* and is critical for maintaining genomic integrity. The KT is a proteinaceous complex that connects centromeric DNA to the spindle. This research focuses on the KMN network – SPC105R, NDC80c, and MIS12c – which forms the outer KT and contains microtubule (MT) binding activity. The KT attachments occur laterally with antiparallel spindle MTs before transitioning to stable end-ons, called KT-MTs (k-fibers). However, the capture of spindle MTs to KTs is error prone, which can lead to mis-segregation of chromosomes. This can result in spontaneous abortions, birth defects, and infertility. We are interested in determining the mechanisms regulating the transition of lateral to end-on attachments and establishing biorientation. KT subcomplex NDC80 is required for end-on attachments and is a proposed target of Aurora B kinase (AURKB) during attachment error correction. Additionally, our lab has previously shown that loss of NDC80 or Protein phosphatase 2A (PP2A) results in primarily lateral attachments. These results show that PP2A is required to dephosphorylate the KT, allowing for NDC80 to establish stable end-on KT-MT attachments. We developed a tool to study both k-fiber dynamics and MT attachments at the KT; *Spc25INbox; Incenp^{RNAi}*. With endogenous *Incenp* knocked down, the *Spc25INbox* transgene targets AURKB to the KT, leading to the formation of a monopolar spindle with only k-fibers. When *Spc25INbox; Incenp^{RNAi}* was paired with *Ndc80*^{RNAi}, we observed lateral attachments, indicating that k-fibers are captured, and not grown by the KT. In contrast, when *Spc25INbox; Incenp ^{RNAi}* was paired with *PP2A^{RNAi}*, we observed end-on attachments, indicating that KT localized AURKB alone is not sufficient for destabilizing KT-MT end-on attachments, and likely relies on a central spindle localized kinase. We propose that central spindle localized AURKB phosphorylates NDC80 and maintains lateral attachments. We have previously shown that lateral attachments are sufficient for chromosome movement. Future work will include experiments to determine the direction of lateral attachment movement, which motor proteins are involved, and determine the relationship between lateral attachments, chromosome movement, and biorientation.

826F **The Use of *Drosophila* Fecundity Measurements as a New Approach Methodology to Identify Reproductive Toxicants** Keezean Paguio[1], Isabella Estevez[2], Cristy Mendoza[3], Raymond Esquerra[2], Todd Nystul[4] [1]Biology, San Francisco State University, [2]San Francisco State University, [3]UCSF, [4]UC San Francisco

A wide variety of industrial chemicals and pollutants are already present in our daily environment and, by some estimates, an average of three new chemicals are introduced every day. In most cases, there is little to no information about the impacts of these chemicals on human health and the ecosystem, so there is an urgent need for approaches that will help determine which chemicals may pose the greatest risk. This need has prompted a call for New Approach Methodologies (NAMs) that are inexpensive, efficient, and avoid the use of vertebrate animal models. In line with this call, we developed a high-throughput method for exposing adult *Drosophila* to specific chemicals under defined conditions and measuring the impact on fecundity. Our method combines a multiwell fly culture strategy with a 3D-printed fly transfer device, a modified 3D printer called RoboCam that captures images of each well, and an image segmentation pipeline that automatically counts the number of eggs in each well. We are using this approach to screen a list of 100 chemicals that have been identified as potential reproductive toxicants based on environmental surveys and assays performed in *Saccharomyces cerevisiae*. We plan to follow up on candidates identified in the initial screen with additional studies in Drosophila and mammalian organoid cultures. In addition, we are working on improvements to the high-throughput fecundity assay to increase efficiency and incorporate methods to detect meiotic defects. Overall, our goal is to establish Drosophila as one link in a robust pipeline consisting of multiple experimental systems to identify reproductive toxicants in the environment.

827F **Regulation of spermatogenesis by Notch signaling.** Emma O›Flaherty, Adrianna Soriano, Christine Severude, Jennifer Jemc Mierisch Loyola University Chicago

The Notch signaling pathway plays a significant role in gonad development and spermatogenesis, but little is known about its specific targets in the testis. The *Drosophila* testis contains two populations of stem cells: germline stem cells and cyst stem cells, which give rise to differentiating germline and somatic cyst cells, respectively. Notch signaling is activated in the somatic cyst cells by the Delta ligand from the germline in a region of the testes coined the "transition zone", where somatic cyst cells progress from early cyst cells surrounding gonialblasts and 2-cell germline cysts to late cyst cells surrounding 16-cell spermatocyte cysts. We have found that increased Notch signaling in somatic cyst cells prevents their complete transition from early to late cyst cell fate, leading to an arrest in late spermatogenesis and sterility. These results suggest Notch signaling is required to enter the transition state, but too much Notch signaling inhibits a complete transition to the late cyst cell fate. To explore the downstream effectors through which Notch mediates these effects, RNA sequencing was performed on testes overexpressing activated Notch and 407 potential Notch targets were identified. Through genetics and gene expression analysis, we identified two members of the *Enhancer of Split Complex* (*E(spl)m3*-bHLH and *E(spl)mBeta*-bHLH) and *groucho* as downstream targets of the Notch signaling pathway in spermatogenesis. To investigate the direct effects of *groucho* activation in the testes, we expressed overactive Groucho protein in the somatic cyst cells using the Gal4/UAS system. Overexpression of activated Groucho resulted in a delayed onset of the transition zone and delayed end of the transition zone. We also observed a decrease in the number of transition zone cells when Groucho is overexpressed relative to the control. These results suggest too much Groucho results in continued repression of Notch targets in somatic cells, preventing the transition of somatic cells from early to late cyst cell fate. Preliminary results of knocking down *E(spl)m3*-bHLH and *E(spl)mBeta*-bHLH expression reveal a delayed onset of the transition state, suggesting that Notch activation of these targets is required to promote the transition state. Our results support a model in which Notch signaling is required for the transition state but must be turned off for somatic cells to completely transition to the late cyst cell fate to promote the late stages of spermatogenesis.

828F **The *Drosophila melanogaster* TENT5 homolog is required for individualization of spermatids during spermatogenesis** Abdulqater Al-Nouman[1], Kyle Helms[2], Jennifer Curtiss[1] [1]Biology, New Mexico State University, [2]Department of Neurology, Columbia University Irving Medical Center

TENT5s are non-canonical poly(A) polymerases (PAPs) that add non-templated adenines to mRNAs post-transcriptionally. Orthologs of TENT5 are important for reactivation of translationally repressed mRNAs in oocytes and in dendrites of neurons and are required for long term memory. Human TENT5s have been implicated in multiple diseases and cancers suggesting a tumor suppressive role. TENT5D has been linked to human male infertility and TENT5C has also been shown to be essential during spermatogenesis in mice. However, the mechanisms by which TENT5s polyadenylate mRNAs remain elusive and they may have other biochemical roles besides poly(A) polymerase activity.

Drosophila melanogaster has one *TENT5* ortholog, *isep*. We have generated a loss of function allele of *isep* and have demonstrated that homozygous males are sterile with defects arising during spermatid individualization. Individualization is the last stage of spermatogenesis where a syncytium of 64 spermatids must be resolved to encase each spermatid in its own plasma membrane and strip away unneeded organelles and cytoplasm. Restricted localization of caspase-3 and synchronous actin cone progress along the axoneme is disturbed in *isep* loss of function. *Isep* is also required for localized accumulation of *scotti*, a key regulator of individualization. Our *in situ hybridization* reveals that *isep* is transcribed in two distinct stages: during the miotic division of spermatocytes and post-mitotically in elongating spermatids where it localizes in a "comet" pattern. Our ongoing work aims to identify the mechanism by which *isep* operates by identifying its mRNA targets and RNA binding protein cofactor(s) in the context of spermatogenesis which will shed light about the elusive mechanisms by which its orthologs may operate.

829F **Regulation of spermatogenesis by the E3 ligase Mindbomb2 and Combover** Carihann M. Dominicci-Cotto[1], Josefa Steinhauer[1], Andreas Jenny[2,3] [1]Develomental and Molecular Biology, Albert Einstein College of Medicine, [2]Albert Einstein College of Medicine, [3]Genetics, Albert Einstein College of Medicine

A higher species' survival requires proper gamete production and differentiation. Sperm count in men has decreased significantly over the last 50 years. Therefore, understanding the signaling pathways that regulate sperm production and fertility is crucial for humanity's survival. As in mammals, during spermatogenesis in Drosophila melanogaster, syncytial spermatids differentiate and individualize into mature sperm tightly enclosed by a plasma membrane. Specialized actin cones move along the sperm tails to separate spermatids during individualization. In this process, inter-spermatid bridges and the excess of the cytoplasm are removed, a prerequisite for sister spermatids to become individual, functional sperm. Our laboratory previously showed that Combover (Cmb), a member of the family of intrinsically disordered proteins and an effector of planar cell polarity under the control of Rho kinase (Rok), is essential for sperm individualization and fertility in Drosophila. We show that CRISPR-generated rok mutants (Crispants) that specifically remove Rok from germline cells cause sterility in male flies. The rok Crispants also have shortened testes, an increase in defects of individualization complexes, and reduced elongation complexes with Cmb at the end of growing axonemes, suggesting that Rok regulates Cmb in the Drosophila germline. To further understand Cmb function in spermiogenesis, we decided to study the role of Mindbomb 2 (Mib2), an E3 ubiquitin ligase that physically interacts with Cmb. Here, we show that Mib2 hypomorphic mutants are partially male sterile, similar to the expression of Mib2 RNAi in the germline. Based on the mutually exclusive localization of Cmb and Mib2 to growing axonemes and elongation complexes, current studies focus on determining if Mib2 targets Cmb for degradation in the sperm tail to promote the transition between elongation and individualization of spermatids.

830F **Evolutionary and genetic investigation of male molecular control of *Drosophila melanogaster* mating plug ejection timing** Jolie A Carlisle, Bianca M Villanueva, Mina Jannatul, Katelyn Boese, Andy G Clark, Mariana F Wolfner Molecular Biology and Genetics, Cornell University

J.A. Carlisle, B.M. Villanueva, J.F. Mina, K. Boese, A.G. Clark, M.F. Wolfner

In many internally fertilizing animal species, ejaculate coagulates within the female to form a hardened mass that contains sperm, seminal fluid proteins (Sfps), and other molecules. In mammals, this structure is called the copulatory plug; the analogous structures in *Drosophila melanogaster* and other invertebrates are called mating plugs. These plugs are believed to facilitate sperm storage and influence sperm competition outcomes. The proteomic composition of the *D. melanogaster* mating plug (MP) has been described in two previous studies and contains both male and female reproductive tract-derived proteins. The timing of D. melanogaster MP ejection is associated with effects on sperm storage levels which could influence sperm competition outcomes. Therefore, the composition of the MP and its influence on MP ejection timing is a potential focal point of sexual conflict over control of paternity outcomes. We use targeted genetic techniques to investigate the individual contributions of male reproductive tissues and individual seminal fluid-derived MP proteins on MP ejection timing. We show that sperm themselves do not affect MP ejection timing but that some individual Sfps do. Finally, we discovered that three Sfps critical for MP function arose recently in *Drosophila*, indicating that the *D. melanogaster* MP is likely molecularly distinct from post-copulatory plugs formed in other clades. The rapid turnover of proteins in the mating plug, along with accelerated substitution rates in some MP proteins, further highlights the role of natural selection mediating MP function.

831F **Mei-P26: The Germ Cell Gatekeeper that even a Western diet can't ignore** Shallinie THANGADURAI, Elena S. Pak, Alexander K. Murashov CBS, Louisiana State University

Mei-P26 is a conserved RNA-binding protein in Drosophila that plays a crucial role in regulating various cellular processes, including meiotic differentiation, transcriptional activation and repression, as well as microRNA (miRNA) regulation. In a previous study, we observed that Mei-P26 was upregulated in the brains of offspring following a paternal Western diet. To investigate how Mei-P26 is epigenetically transmitted to the offspring, we examined the germ cells of fathers on the Western diet. We found an increase in Mei-P26 mRNA levels in the sperm. Although it is well established that most mRNAs are degraded during the histone-to-protamine transition, recent findings suggest that sperm carry long RNAs, including mRNAs and lncRNAs. We hypothesize that the presence of Mei-P26 in sperm may suppress the RNAi machinery until fertilization. Our results also reveal the intricate relationship between Mei-P26 and miR-1006 in modulating key cell cycle regulators, Cyclin A and Cyclin B. Through loss- and gain-of-function approaches in Drosophila adult gonads and brains, we show that Mei-P26's involvement in miRNA-mediated repression of cell cycle regulators is essential for proper progression through the G1/S transition. Additionally, overexpression of Mei-P26 in germ cells resulted in cell cycle arrest during the S phase, leading to the formation of truncated testes and underdeveloped ovaries. In somatic cells, overexpression of Mei-P26 caused lethality and tissue overgrowth in the gonads. Together, our findings provide new insights into how post-transcriptional regulation by Mei-P26 and miRNAs coordinates cell cycle events, potentially influencing developmental timing and cellular growth control. These results also suggest that Mei-P26 plays a critical role in determining the fate of germ cells and that epigenetic alterations in this process could impact offspring phenotype.

832F **Dissecting the role of Mlp60A in the development and reproduction of *Drosophila melanogaster*** Rounab Sarkar Department of Developmental Biology and Genetics (DBG), Indian Institute of Science

The motifs and domains specific to a protein mediate its distinct biological functions. The LIM (Lin11, Isl-1, and Mec-3) domains present in various nuclear and cytosolic proteins are essential to protein-protein interactions that support various biological activities. The low redundancy of LIM domain proteins in Drosophila makes it an excellent model to address novel functions of such proteins. The Drosophila Muscle LIM Protein at 60A (Mlp60A) and its homologs are essential in myogenic differentiation and cardiac and skeletal muscle development. Even though MLPs are considered important proteins for muscle development, the activities of Mlp60A and its homologs in non-muscle cells have not yet been understood. Unpublished data from our lab and the transcriptomic analysis of *Mlp60A* suggest that Mlp60A may have an effect on reproduction. Therefore, this study aims to identify the non-muscle roles of Mlp60A in reproduction. Our data indicates that $Mlp60A^{null}$ flies have compromised reproductive functions, including oogenesis and spermatogenesis and that Mlp60A may exert a maternal effect on embryonic development. Moreover, follicle cell-specific knockdown of *Mlp60A* affects female fertility and fecundity by disrupting the morphogenesis of the follicular epithelium. Our results further suggest that Mlp60A is crucial for border cell migration and specification. Here, we identify that Mlp60A is important in maintaining the population of somatic cyst cells during spermatogenesis, with cyst cell-specific knockdown of *Mlp60A* causing a reduction in male fertility. To reveal the regulatory mechanisms of Mlp60A in *Drosophila* oogenesis and spermatogenesis, we have identified a reciprocal negative regulatory relationship between *Mlp60A* and the JAK/STAT and EGFR signaling pathways. This interplay is crucial for maintaining the proper morphological development of both follicle cells and cyst cells. Our results further suggest that Mlp60A is pivotal in regulating JAK/STAT signaling for border cell migration and specification. Our findings provide insights into the non-muscle function of a crucial muscle protein, MLP, in reproduction, particularly in the regulation of somatic cells (follicle cells and cyst cells). Additionally, we propose border cells as a model to understand the function of LIM proteins in cell specification and migration.

Keywords: Drosophila, Oogenesis, Spermatogenesis, Mlp60A, JAK/STAT, Cell migration, Cell specification, Border cells, Follicle cells

833F **Phenocopying the spermatid individualization phenotype of *mulet* by ectopic germline expression of Tubulin-folding Cofactor E (TBCE)** James J Fabrizio, Simone S Caruso, Emily J Maestre, Susan J Rodriguez Natural Sciences, University of Mount Saint Vincent

Spermatogenesis in all animals occurs within a germline syncytium, and mature sperm are resolved from this common cytoplasm post-meiotically during spermatid individualization. The individualization process is accomplished by a membrane-cytoskeleton complex known as the individualization complex (IC), which assembles around the sperm heads and travels down the sperm tails, removing excess cytoplasm from between the sperm tails and encasing each spermatid in its own plasma membrane. In order for individualization to be successful, a population of approximately 100 inter-flagellar microtubules must be removed by Tubulin-folding Cofactor E-like (TBCEL), the protein product of the *mulet* gene. Failure to remove these microtubules results in an individualization phenotype where the IC becomes severely disrupted (**Fabrizio et al., 1998, 2012, 2020**). Unlike TBCE-L, TBCE (Tubulin-folding Cofactor E) promotes the synthesis of microtubules. Thus, we hypothesized that over-expression of TBCE in wild-type testes would promote the retention of inter-flagellar microtubules during spermatogenesis and phenocopy *mulet*. Indeed, ectopic germline expression of TBCE using *bam-Gal4VP16* produced disrupted ICs characteristic of *mulet* mutant testes. Furthermore, increased GAL4 activity at higher temperatures increased the severity of the phenotype, suggesting a relationship between the degree of inter-flagellar microtubule retention and individualization failure. Thus, the persistence of inter-flagellar microtubules, accomplished either by mutating TBCEL or overexpressing TBCE, produces a severe individualization phenotype. Taken together with previous results from our lab, these data further highlight the necessity of removing these microtubules as a prerequisite for normal spermatid individualization.

834F **Discovering Novel Meiosis Mediating Genes in *Drosophila melanogaster*** Diya Surray[1], Kim S McKim[2], Tia Nissimov[2], Daria Mitri[1], Neha Changela[1] [1]Rutgers University, [2]Genetics, Rutgers University

Formation of gametes relies on the division of germ cells during meiosis. Female meiosis is unique due its long periods of arrest, extended prophase, and being acentrosomal. It is hypothesized that a lack of centrosomes causes oocytes to have increased aneuploidy. Since many of the genes required for oogenesis are still unknown, this project aims to find novel genes implicated in female fertility, with a focus on better understanding chromosome biorientation. Using RNA interference, we have screened through genes expressed in the *Drosophila melanogaster* ovary, and genes that are orthologous to ones mutated in women with high aneuploidy. We are using two Gal4 drivers to knocks down genes either throughout the entire germline, or only in meiotic prophase and metaphase. If ovarian gene knockdown causes sterility or increased aneuploidy, then further study was conducted to understand its meiotic role. Thus far, we have screened 549 genes, of which, 95 caused sterility, and 36 caused high aneuploidy. For the genes that caused sterility, we have examined if these oocytes completed all stages of development and arrested at metaphase 1 (Stage 14), which could give a clue as to gene function. We found that 32 of the 95 genes did not complete development, with many of them halting in prophase. To investigate gene function, we analyzed 11 positive candidates by immunostaining for the DNA, spindle, kinetochores, and central spindle. For example, knock down of *CG18787* caused precious anaphase, elongated spindles, and premature separation of the sister chromatids. This indicates that *CG18787* may be involved in sister-chromatid cohesin. The lack of cohesin to hold the sister chromatids could cause them to segregate early. Additionally knockdown of *CG12259* resulted in increased aneuploidy. Cytological staining showed defects in germ cell development, the synaptonemal complex, and double strand breaks. We are currently testing for decreased crossing over that could tie the lack of double strand breaks to the increased aneuploidy. Also, we have identified cytokinetic genes such as *Rho1* and *sticky*, which are mutated in women with high aneuploidy. We are examining the role of these missense mutations by replicating them in the *Drosophila* orthologs. We have started by making mutations in the gene *Rho1* and are now investigating the impact on fertility in these mutants.

835F **Lipid production in early germ cells maintain oocyte quality during *Drosophila* oogenesis** Bhawana Maurya, Allan Spradling Department of Embryology, Carnegie Institution for Sciences

Drosophila ovarioles produce and export 2-3 new follicles per day that form within an initial “germarium” region containing germline stem cells (GSCs) and niche cells: terminal filament cells (TFCs), cap cells (CCs) and escort cells (ECs). Each follicle is built from a germline cyst comprising 15 nurse cells interconnected to an oocyte by a fusome, a germ cell-specific organelle containing Hts and spectrin proteins and rich in ER. We found that germ cells contain abundant cytoplasmic lipid droplets (LDs) beginning with the GSC that move in association with the fusome especially in region 2b and become enriched in oocytes along with the Balbiani body. Consequently, we studied how lipids are formed in early germ cells and their role during oogenesis.

To assess if TFs and CCs are involved in lipid production and contribute to the lipid pool of germarium, we selectively knockdown genes involved in lipid transport through vesicular trafficking and membrane transporters using *bab-GAL4* driver. Knockdown of Secretory 6 (Sec6) component of exocyst complex and Oatp30B an organic anion transporter, blocked lipid transport from TFs and CCs to GSCs and developing cysts. Further to understand if early germ cells depend solely on TFs and CCs for lipid intake, or in addition they are able to synthesis lipids on their own. We knocked down *hts* using *nanos-GAL4* which disrupted fusome as expected, but also strongly reduced germ cell LDs, suggesting a role for the fusome ER in lipid synthesis. We postulate TFs and CCs serve as an initial source for important lipids in refurbishing germ cells both in their plasma membranes and organelles, and the same role is taken up by fusome in developing cyst. Interestingly we observed that germaria devoid of lipid droplets have elevated level of reactive oxygen species (ROS) and lipid peoxidation, indicating lipid droplets protect early germ cells from lipotoxicity. Thus, during germ cell and follicle development, lipid droplets likely help initiate oogenesis, rejuvenate organelles in transit along the fusome and protect the oocyte from damaging environmental agents.

836F **Orthologs of an essential, lineage-specific spermatogenesis gene vary in their capacities for function and subcellular localization in *D. melanogaster*** Prajal H Patel, Kerry L McDermott, Geoffrey D Findlay Biology, College of the Holy Cross

goddard (gdrd) is a putative *de novo* evolved gene that has become essential for male fertility in *Drosophila melanogaster*. Since the gene's birth at the base of the *Drosophila* genus, the Gdrd protein has become highly divergent across descendant species, with one such lineage even showing gene loss. These observations raise questions about when *gdrd* evolved the essential cellular function observed in *D. melanogaster* and what impacts these evolved sequence differences have had on the function of divergent *gdrd* orthologs. To investigate these questions, we replaced the *melanogaster* coding sequence in a defined, functional rescue construct with codon-optimized, HA-tagged *gdrd* coding sequences from the *simulans*, *yakuba*, *ananassae*, *virilis*, *mojavensis*, and *grimshawi* species. Analyses of the subcellular localization across all orthologs reveals that the protein maintains its capacity to bind axonemes and the ring centriole, suggesting that these interactions arose at the base of the *Drosophila* genus. Despite this conserved subcellular localization pattern, however, the *ananassae*, *virilis*, and *grimshawi* orthologs failed to restore fertility in *gdrd* null *D. melanogaster*. Several mechanisms potentially account for this failure, including protein instability (*grimshawi*) and weakened axonemal binding (*virilis* and *ananassae*). The *yakuba*, *ananassae*, and *mojavensis* orthologs also show novel subcellular distributions when expressed in *melanogaster*, indicating that these proteins may have potentially evolved additional binding partners in their respective lineages. Future work analyzing the functional requirement and the subcellular localization of *gdrd* orthologs in non-*melanogaster* species will inform us definitively about the evolution of this lineage-specific gene.

837F **Lagging strand DNA polymerases regulate reproductive potential in *Drosophila* Germline** Yijun Liao[1], Brendon Davis[2], Rajesh Ranjam[2,3], Xin Chen[2,3] [1]Biology, Johns Hopkins University, [2]Johns Hopkins University, [3]Howard Hughes Medical Institute

Reproductive success affects survival of animals. In the *Drosophila* germline, the self-renewal ability of germ stem cells (GSCs) is required for sustainable production of both sperms and oocytes. During aging, both male and female flies exhibit significantly reduced fertility, in part due to the decreased GSC division, increased cell death and insufficient regeneration. DNA polymerase alpha and delta (polα and polδ) are responsible for lagging strand synthesis during DNA replication. Besides, Polα helps maintain epigenetic information. Previously we found several DNA replication machinery components, including Polα and Polδ, have reduced expression levels in male GSCs compared to transit-amplifying spermatogonial cells(SGs). Particularly, reducing Polα levels or activities in SGs can induce local asymmetric histone incorporation during DNA replication (Snedeker et al., 2024). However, the biological importance of this phenomenon was unclear. By halving protein levels using heterozygous(het) flies, we discovered that both polα and polδ het male and female flies show significantly higher fertility than control over time, without compromising their longevity. We hypothesize that this enhanced reproductive longevity is promoted by better GSC maintenance and/or enhanced progenitor cell reprogramming. In male germline, when the GSCs are lost from aging or injury, progenitor SGs could undergo dedifferentiation and become GSC-like cells. However, these GSC-like cells are normally dysfunctional for they carry misoriented centrosomes and are arrested for entering mitosis. Meanwhile, in both male and female, signaling activities which activate downstream transcription factors STAT92E or MAD to maintain GSC identity, decline during aging. In aged polα het males, we found a significant higher percentage of GSCs with properly oriented centrosomes. These GSCs have higher level of STAT92E expression, with a more normal testes hub structure and overall testicular morphology. In polα het females, GSC number are better maintained than control during aging, with stronger pMAD signals across all time courses. In addition to higher stem cell activities, polα het males display improved regeneration when the GSCs are genetically ablated, where the de-differentiated GSCs regain more *bone fide* GSCs features than control. In conclusion, our work revealed that DNA replication components contribute to regulating GSC maintenance, GSC activity, and the regeneration potentials in the germline lineages of *Drosophila*. This finding provides a new therapeutic approach for promoting regeneration and elongating reproductive longevity.

838F **The role of Cpsf5 and Cpsf6 in maintaining germline stem cells and regulating oogenesis of *Drosophila*** Yu-Te Lan[1], Shih-Wei Chen[2], Jenn-Yah Yu[3] [1]Interdisciplinary Master Program in Molecular Medicine, National Yang Ming Chiao Tung University, [2]National Yang Ming Chiao Tung University, [3]Department of Life Sciences and Institute of Genome Sciences, National Yang Ming Chiao Tung University

Many eukaryotic genes contain multiple polyadenylation (poly(A)) signals (PAS) that may undergo alternative polyadenylation (APA), resulting in different 3' untranslated regions (UTRs). The 3'UTR is a crucial platform for interacting with microRNAs and RNA binding proteins, thereby affecting mRNA stability, localization and efficiency of translation. The cleavage factor I (CFI) complex components Cpsf5 and Cpsf6 are shown to regulate APA, but their physiological functions are not well understood. In this study, we used the *Drosophila* ovary as a model to investigate the roles of Cpsf5 and Cpsf6 *in vivo*. In *Cpsf5* trans-heterozygous mutants, the endocycle of nurse cells were disrupted and follicle cells became multi-layered. The growth of oocyte and egg chamber was stalled at stage 8 and no mature egg was observed in *Cpsf5* trans-heterozygous mutant ovary. bag of marbel (bam), which should be suppressed in the germline stem cell (GSC), was ectopically expressed in *Cpsf5* trans-heterozygous mutant GSCs. I further used the Flp-FRT system to generate GSC clones mutant for *Cpsf5* or *Cpsf6*. Phosphorylation of Mad, which indicate the activity of the BMP signal, was decreased in *Cpsf5* or *Cpsf6* mutant GSCs. Importantly, *Cpsf5* or *Cpsf6* mutant GSCs were quickly lost from the niche. Together, our data suggests that Cpsf5/6 are crucial for GSC maintenance and oogenesis. We use 3'RNA sequencing and search for candidate genes undergo Cpsf5/6-mediated APA. Currently, we are analyzing our 3'RNA sequencing data.

839F **Evaluating Directional Cues That Affect Migratory Preference In Border Cell Migration** Elana Frazier, Alexander George, Christopher Welsh, Michelle Starz-Gaiano Biological Sciences, University of Maryland, Baltimore County

Collective cell migration is vitally important to development, wound healing, and cancer metastasis. Characterizing the signaling pathways and regulation of this process is crucial to the advancement of current therapies. To do this, we study a collective cell migration observed during Drosophila melanogaster oogenesis, within the tissue that gives rise to the egg, the egg chamber. The border cell cluster (BCC) detaches from the anterior follicular epithelium and migrates posteriorly toward the oocyte, extending membrane protrusions in the direction of migration. This migration is guided by chemoattractants and physical interactions with egg chamber architecture. Additionally, chemoattractant signaling alters both directionality and stability of BCC protrusions. How directional cues and architecture interact to regulate BCC migration is still unclear. Using genetic tools, we induced mosaic expression of an oncogenic pathway component to produce BCCs in ectopic locations. Then we analyzed directional preferences of BCCs within novel migration paths. Quantification of the directional selection of protrusions suggests ectopic BCCs have a preference for specific tissue structures along the migration path and indicates potential distribution patterns of chemoattractants. Heparan sulfate proteoglycans (HSPG) have been shown to modify the distribution of extracellular signaling molecules, including chemoattractants, and we hypothesize that they have the same function in regulating BCC migration. The tout velu family of genes involved in the biosynthesis of HSPGs interacts with multiple signaling pathways, but its role in BCC migration has not been characterized. We used RNA interference to reduce expression of tout velu (ttv), sister of tout velu (sotv), and brother of tout velu (botv) in both the BCC and their substrate cells, which led to defects in BCC specification and cohesive movement. We predict that these defects will be exacerbated by the genetic reduction of extracellular signals that interact with this pathway, which we are currently testing. This project provides insight into mechanisms that guide collective cell migration in diseases like metastatic cancers, which migrate in response to diverse chemical cues and physical interactions.

840F **Juvenile hormone signaling is sex-specific and highly dynamic throughout gonad development** Krystal Goyins, Lia Sorrell, Samantha Cocita, Harry Siegel, Lacy Barton University of Texas at San Antonio

Sexual reproduction requires a precise balance and timely fluctuation of systemic hormones. How hormones are locally regulated within ovaries and testes remains an open question. In Drosophila and other insects, retinoid-like small molecules called Juvenile hormones (JHs) are important for female and male fertility, yet specifically *where* and *when* signaling is active in development and adult gonads, as well as how JHs support fertility remains unclear. To close this knowledge gap, we generated fluorescent JH reporters to identify gonadal cell types active for JH signaling. We found that JH signaling is prevalent in the testis germline stem cell niche hub cells throughout larval, pupal and adult stages. In contrast, JH signaling is highly dynamic in the developing ovary such that different cell types exhibit active JH signaling depending on the stage of ovarian development. In the larval ovary, JH signaling is active in swarm cells and terminal filament cells. Interestingly, JH signaling is notably absent from the pupal terminal filaments and is instead observed only in the basal stalk cells in pupal ovaries. In the adult ovary, JH signaling is active in the escort and follicle stem cells. These data demonstrate that JH signaling is spatially restricted to subsets of male and female gonadal cells despite systemic JH circulation. We hypothesized that spatiotemporal restriction of JH signaling is shaped by JH degradation enzymes. To test this hypothesis, we generated single, double, and triple Drosophila mutant strains lacking a key JH synthesis enzyme and two classes of JH degradation enzymes. Using a sperm exhaustion assay to measure male fertility of these JH mutants, we found JH synthesis is required for male fertility as expected. We also found that loss of one JH degradation enzyme class reduces male fertility, while loss of the other class of JH degradation enzyme class prolongs male fertility compared to wild type controls. In contrast, loss of either class of JH degradation enzymes compromises female fecundity. Together, these data suggest that precise control of JH titer or spatial activity is required for gametogenesis in both sexes. Moving forward, we will determine *how* JH contributes to male and female fertility by identifying JH target genes in the gonadal cell types with active JH signaling. We hope results from this work will reveal how systemic hormones are locally regulated to drive animal reproduction.

841F **Ovarian germ cells use EcR to stimulate timely cyst packaging** Lindsay Swain, Elizabeth Ables East Carolina University

Oocytes are carefully packaged into ovarian follicles, each containing a maturing germ cell surrounded by a layer of somatic cells that secrete protective eggshell and chorion proteins. This conserved arrangement is essential for proper oocyte development and reproductive success. Continuous coordination and bi-directional signaling from somatic cells to germ cells is necessary for proper oocyte packaging. In *Drosophila*, although germ cell packaging was presumed to be largely controlled by somatic follicle cells enveloping passive germ cells, recent studies suggest that germ cells themselves produce motor forces that drive somatic encapsulation. Here, in support of this hypothesis, we present data suggesting that cyst encapsulation is dependent upon Ecdysone Receptor, a steroid hormone receptor known to control multiple aspects of oogenesis. Using tools to deplete EcR levels or block transcriptional activation specifically in the germline, we show that germline-autonomous EcR is necessary for the timing of cyst encapsulation. In the absence of EcR, germ cell encapsulation is slowed, resulting in increased incidence of cyst collision events in the germarium. EcR facilitates germ cell cyst encapsulation by signaling somatic cells to promote proliferation and suppress pre-stalk cell gene expression. Overall, these data suggest that in addition to its well-characterized roles in somatic follicle cells, EcR is necessary in the germline to promote ovarian follicle assembly and development.

842F **Subcellular Localization of Vacuolar ATPase During Ovarian Cell Death in *Drosophila melanogaster*** Logan Tohline[1], Shruthi Bandyadka[2], Kim McCall[2] [1]Graduate School of Arts and Sciences, Boston University, [2]Boston University

During oogenesis in *Drosophila melanogaster*, germline cell death outside of the germarium occurs primarily at two points: apoptosis during mid-stage development, in response to unfavorable environmental stimuli such as starvation, and phagoptosis during normal late-stage development, when nurse cells transfer their cytoplasm into the oocyte and are removed by surrounding stretch follicle cells. Vacuolar ATPase (V-ATPase) transmembrane proton pumps in the plasma membrane of follicle cells have previously been shown to drive the acidification of the extracellular space during late-stage phagocytosis, and thus are critical for cell clearance. However, V-ATPase is not ubiquitously found throughout the follicle cell plasma membrane during all stages of oocyte development. Rather, it appears to localize differently in healthy egg chambers and during cell death, and depends upon the type of follicle cell. This research works to elucidate the dynamic details of subcellular V-ATPase localization throughout oogenesis, especially during mid- and late-stage germline cell death.

843F **The neurodegeneration gene *iPLA2-VIA* is required in GABAergic neurons for mitochondrial maintenance in the *Drosophila melanogaster* female germline** Aryeh Levenbrown, Philip Hirschprung, Eliezer Heller, Samuel Intrator, Sarah Liberow, Josefa Steinhauer Yeshiva University

Loss of function mutations in the gene *PLA2G6*, encoding the group 6A calcium-independent phospholipase A2, are associated with severe neurodegenerative disease in humans, including neuroaxonal dystrophy and autosomal recessive parkinsonism. The orthologous *Drosophila melanogaster* gene *iPLA2-VIA* encodes a highly similar protein, and loss of function mutations in flies lead to neuronal death and age-dependent locomotor defects. In *Drosophila,* loss of *iPLA2-VIA* additionally leads to reduced female fertility and age-dependent defects in the female germline, including mitochondrial aggregation, loss of mitochondrial membrane potential, and eventual germ cell apoptosis. We explored the tissue autonomy of the germline defects using our recently developed method to quantitatively analyze nurse cell mitochondrial aggregation. Surprisingly, ubiquitous somatic knockdown led to strong mitochondrial aggregation and death of female germ cells, suggesting a critical non-autonomous component to the germline effects. Further experiments showed that *iPLA2-VIA* knockdown in neurons, and specifically in GABAergic neurons, led to very strong germline defects, phenocopying the knockout mutant. Feeding assays indicated that the germline defects produced by neuronal knockdown were not simply a byproduct of starvation in the knockdown flies. Our findings suggest an unanticipated role for neuronal maintenance in germ cell health during aging, with a particularly important role for *iPLA2-VIA* in the GABAergic neurons.

844S **Characterizing the effects of the overexpression of Eip75B in *Drosophila*** Allison C Simmons, Alexandria I Warren, Elizabeth T Ables Biology, East Carolina University

Nuclear hormone receptors link nutritional signals to cellular responses necessary for development, reproduction, and viability. In *Drosophila*, two nuclear receptors, *Ecdysone Receptor* (*EcR*) and *Ecdysone-induced protein 75B* (*Eip75B*) are transcribed in response to the steroid hormone ecdysone and genetically interact to regulate developmental transitions and reproduction. Characterized by a unique N-terminal sequence, Eip75B has three protein isoforms (Eip75B-A, Eip75B-B, and Eip75B-C). Eip75B-B lacks a DNA binding domain, but heterodimerizes with Hormone receptor 3 (Hr3), an orphan nuclear receptor. Depending on the cellular context, Eip75B functions as either a transcriptional target of EcR or a repressor of EcR-responsive genes. Yet while both are expressed and necessary for proper oogenesis, their molecular relationship is unclear. Previous studies found that loss of *Eip75B* increased germline stem cell maintenance and arrested egg chamber development during mid-oogenesis, and isoform-specific over-expression resulted in egg chamber apoptosis. How Eip75B interacts with EcR in the ovary is unknown. To begin to address this question, we created two novel transgenes that over-express *Eip75B-A* or *Eip75B-B* under the control of upstream activating sequences (UAS) that can be driven in either the germline or the soma. We found that over-expression of either isoform in all somatic cells promotes premature lethality. Using these transgenes, we will comprehensively over-express *Eip75B* in the ovarian germline and somatic cells using a variety of ovarian Gal4 drivers. We hypothesize that the over-expression of the isoforms in the germline will cause flies to be agametic. We anticipate that these results will assess potential independent roles in oogenesis, and lead to future studies dissecting the interplay between Eip75B and EcR.

845S **Characterization of *CG34168 i*n fly wing and sperm development** Karah Mayer, Safoora Syeda, Zollee Williams, Jennifer Hackney Arizona State University

In *Drosophila melanogaster*, the mid-third instar transition (MIT) is a developmental time point at which widespread changes in gene expression occur, likely serving to prepare the animal to enter metamorphosis. A subset of genes that are upregulated at the MIT display an unusual expression profile, with expression limited to imaginal discs in larvae at the MIT, and testes of adult males. Most of these genes are uncharacterized. We investigated the potential functions of one of these genes, *CG34168* in wing development and male fertility. Wing development (wing size and trichome density) and male reproduction (testis morphology, sperm production, and fertility) were examined in *CG34168*^{f02253} and *OregonR* flies. This work further elucidates the function of one of many uncharacterized MIT genes expressed in seemingly unrelated tissues (wing and testes) and contributes to an understanding of molecular mechanisms influencing wing development and male fertility.

846S **Abnormal crossover patterning in flies deficient in an X chromosome boundary site** Ilan Socolovsky-Hull, Susan McMahan, Jeff Sekelsky Biology, University of North Carolina at Chapel Hill

Chromosomal aberrations result in disruptions to meiotic recombination patterning, with lower recombination rates near the translocation breakpoints (Hawley, 1980). This reduction in recombination is not distance dependent, and the effect instead seems be limited to within one of several sections of the chromosome. On the Drosophila X chromosome, each of these 5 sections is bounded by regions of intercalary heterochromatin, and recombination rates return to near-wildtype levels beyond boundary sites. To investigate the nature of these boundary sites, we measured recombination using genetic marker screens in female flies heterozygous for deficiencies spanning the 11A boundary site. We found that while all female flies heterozygous for deficiencies near the 11A boundary site had reduced recombination rates between *v-g* (9F11-12B4), those carrying deficiencies spanning the 11A boundary site have double the rate of recombination in *v-g* than those with deficiencies not spanning the boundary site. To map boundary sites at high resolution and the effects of their removal, we will collect sons of Df/marker heterozygous females. Since Df/Y is lethal, these progeny will carry the marker chromosome at the position of the deletion, but recombination will result in an increasing proportion of sons carrying deficiency chromosome as distance increases from the deletion. This recombination will occur at a frequency dependent on both the distance from and any regional effects of the deletion. Sequencing sons en masse and plotting frequency of SNPs from the marker chromosome (Wei, 2020) as a function of distance from the deletion should reveal boundary locations at high resolution.

847S **Mitochondria-translation axis promotes epigenetic silencing of germ cell genes to promote transition of germ cells to an oocyte during Drosophila** Anupriya Ramamoorthy[1], Lina Seojin Park[1], Vernon Monteiro[2], Deepika Vasudevan[3], Thomas Hurd[2], Prashanth Rangan[1] [1]Icahn School of Medicine at Mount Sinai, [2]University of Toronto, [3]University of Pittsburgh School of Medicine

The continuity of sexually reproducing organisms hinges on the successful differentiation of germ cells, which undergo meiosis to form oocytes. Upon fertilization, these oocytes initiate the next generation and provide essential mRNAs and protein, known as the maternal contribution, that support early embryonic development. Using Drosophila oogenesis as a model system, we have identified a critical transition in germ cell differentiation that we termed the germ-cell-to-maternal transition (GMT). During GMT, germ cell-specific genes, such as *ribosomal protein S19b* (*rpS19b*), are silenced through heterochromatin formation. The silencing of germ cell genes is concurrent with oocyte specification. To uncover additional regulators of GMT, we performed an RNAi screen assaying for *rpS19b* silencing, using an *rpS19bGFP* transgene as a readout. This screen revealed that key components required for mitochondrial homeostasis, such as Complex V, a mitochondrial protease, and Mitofusin, are essential for proper GMT. Loss of these mitochondrial components results in ectopic expression of germ cell genes like RpS19b within egg chambers, along with defects in oogenesis. We previously observed that mitochondrial dysfunction can activate the integrated stress response (ISR), which suppresses translation to promote stress response gene expression. We examined whether ISR was activated by mitochondrial perturbations. Indeed, loss of mitochondrial components like Complex V led to elevated phosphorylated eIF2a, a marker of translational repression. Independent disruption of translation through reduced TORC1 activity, impaired ribosome biogenesis, or depletion of translation elongation factors resulted in ectopic RpS19bGFP expression and disrupted GMT. Polysome sequencing of these translation-disrupting mutations revealed downregulation of multiple factors involved in heterochromatin formation, implicating translation as a critical link between mitochondria and the silencing machinery for GMT. Thus, our findings discover a mitochondria-translation axis that promotes GMT.

848S **Phospholipase C 21C is required for Primordial Germ Cell migration** Lorena Roa-de la Cruz, Creehan Healy, Calli E Raver, Lacy J Barton The University of Texas at San Antonio

The health of sexually reproducing animals requires proper development of the germline. Across the animal kingdom, sperm and egg precursors, called Primordial Germ Cells (PGCs), are specified early in embryonic development and must migrate through many tissues to colonize the somatic gonad. This PGC journey is essential for fertility and failure to properly colonize the gonad risks the formation of extragonadal germ cell tumors in humans. Our lab uses *Drosophila melanogaster* to discover genetic and non-genetic factors in PGC migration. Following up on our recent findings that retinoic acid-like Juvenile hormones are both necessary and sufficient for PGC migration, we found that Phospholipase C at 21C (Plc21C) genetically interacts with Juvenile hormone biosynthesis enzymes to support PGC migration. Using heteroallelic mutations, we found that global, maternal and zygotic loss of Plc21C significantly compromises PGC migration. Mesodermal-specific knockdown of Plc21C using three distinct RNAi lines revealed Plc21C is not required in the mesoderm, together suggesting Plc21C is autonomously required in PGCs for their migration to the gonad. Plc21C is stimulated by G protein-coupled receptors inducing downstream calcium signaling. To determine if calcium signaling is active in migrating PGCs, we combined the germline-specific calcium sensor transgene, nanos-GCaMP6 (Hu & Wolfner, 2019), with the germline-specific Lifeact-tdTomato transgene (Lin, Luo & Lehmann, 2020). Using spinning disk confocal microscopy in live embryos, we found that Drosophila PGCs initiate asynchronous calcium pulses once they enter the mesoderm, marking active migration to the somatic gonad. We are now completing targeted genetic, pharmacological and subcellular localization screens to identify factors required upstream and downstream of Plc21C in PGC migration. Thus far, we found that inositol 1,4,5-trisphosphate (IP3) is required within PGCs for their migration and that Stromal Interaction Molecule, STIM, required for store-operated calcium entry, is polarized in migrating PGCs. Together, these findings suggest that, like in zebrafish and chicken (Blaser et al., 2006; Morita et al.,2023), calcium signaling is required for PGC migration in Drosophila. We anticipate further investigations using the Drosophila model will shed light on the conserved requirements for calcium signaling in PGC migration.

849S **Investigating the Impact of I element Activation on Meiotic Recombination** Diane Nguyen, Justin Blumenstiel University of Kansas

Transposable elements (TEs) are mobile genetic elements that can move within the genome, and their activation can have a significant impact on genome stability and reproduction. In *Drosophila*, hybrid dysgenesis occurs when males carrying active TE families fertilize females lacking these elements, resulting in reduced fertility. The I-R hybrid dysgenesis system in *Drosophila melanogaster* is a well-established model for studying this phenomenon, where the activation of the I element, a non-LTR retrotransposon, leads to catastrophic meiotic defects and the failure of embryo hatching. In previous studies using *D. virilis*, the activation of a DNA transposon early in development was linked to increased mitotic recombination, but no changes were observed in the meiotic recombination landscape. This may be because in *D. virilis*, transposon activation occurs early during germline stem cell development, before meiosis, and does not perturb meiotic processes directly. In contrast, the I element in the *D. melanogaster* I-R system is activated during meiosis, allowing for a more direct investigation of its effects on meiotic recombination. We are particularly interested in understanding how the activation of TEs influences the process of gametogenesis, specifically how I element activation affects meiotic recombination. We aim to investigate how parental TE asymmetry in the I-R hybrid dysgenesis system affects the landscape of meiotic recombination in *D. melanogaster*.

851S ***Drosophila* oviposition in Glyphosate-Based Herbicide Contaminated Environments** Amelie Carballo[1], Julia Lambert[2], Sebastian McGee[3], Rushil Nandra[4], Mike Rizzo[3], Erik Johnson[3], Becky Talyn[5,5] [1]University of California, Davis, [2]University of California, Berkeley, [3]Biology, Wake Forest University, [4]Department of Biology, California State University, [5]College of Natural Sciences, California State University

Glyphosate-based herbicides (GBHs) kill weeds around crops. GBHs contaminate food and elicit concerns across animal taxa. We examine how the GBH Roundup Super Concentrate (RSC) affects habitat choice for oviposition behavior, using *Drosophila melanogaster*. Initial experiments investigated whether *Drosophila* avoid laying eggs in GBH contaminated sites. Groups of 30, mature female flies reared on a yeast diet in mixed-sex groups were introduced into a partitioned petri dish that contained 4 concentrations of RSC in food medium: control (0), 2, 5, and 10g/L in an embryo collection chamber. Each 3-hour trial was left in the dark to encourage oviposition. Surprisingly, flies laid most eggs in the highest concentration of glyphosate in 87.5% of trials. Mixed plates containing agar (non-nutritive substrate), uncontaminated food medium, 10g/L, and 20g/L of RSC were used in the same manner. In 72.2% trials, flies lay most eggs in the RSC-containing quadrants. Finally, using female *orco* flies, a mutation that deactivates the flies' olfactory receptors, helps determine whether olfactory perception influences response to RSC. Preliminarily, *orco* females lay dramatically fewer eggs overall and do not demonstrate a clear preference for any treatment. In a separate experiment using 2-chamber plates and higher GBH concentrations in grape agar, flies laid more eggs in control medium. Anti-microbial properties of RSC and glyphosate may explain the unexpected preferences, with females preferring to lay eggs in habitats with fewer bacteria. Our future work will examine if substrates mimicking bacterial activity (LPS) deter oviposition behavior of *Drosophila*.

852S **Development of High Throughput Cloning Strategies in *Drosophila melanogaster* to Investigate Reproductive Gene Function** Samantha Valeiron[1], Yasir Ahmed-Braimah[2], Anne Scuderi[2] [1]Syracuse University, [2]Biology, Syracuse University

Reproduction is fundamental biological process, and understanding the genetic basis and molecular mechanisms of reproductive interactions is key to our understanding of evolution and biodiversity. Reproductive traits evolve rapidly between species, and while we have extensive conceptual understanding of the evolutionary processes that drive this rapid divergence, our understanding of this process at the molecular level remains superficial. Male reproductive proteins have been characterized in far more depth in *Drosophila melanogaster*, which is the best genetic system to investigate complex reproductive interactions that are broadly conserved, but female reproductive proteins are largely unexplored. Thus, there exists a large gap in our knowledge of the nature of reproductive interactions because our understanding of the female component is lacking. My research aims to accomplish two tasks: (1) developing a high throughput strategy for creating targeted gene edits (null mutations and allelic replacements), and (2) deploying this high throughput strategy in a set of proof-of-principle experiments to characterize the functions of highly conserved and rapidly evolving female reproductive genes. Here I demonstrate the use of this modular system that combines liquid-handling robotics with high-throughput cloning design and implementation to generate reagents for CRISPR-mediated characterization of female reproductive genes. I also present results on the functional characterization of several genes that are specifically expressed in the female reproductive tract of *D. melanogaster.*

853S **Investigating How Oocyte Age Impacts B Chromosome Transmission in *Drosophila melanogaster*** Annette R St Jacques[1], Stacey L Hanlon[2] [1]Molecular Cell Biology, University Of Connecticut, [2]Molecular Cell Biology, University if Connecticut

B chromosomes are nonessential chromosomes that are carried in addition to the essential chromosome set. Although B chromosomes are not required for the normal growth or reproduction of an organism, they can persist and accumulate in populations through non-Mendelian inheritance mechanisms. Recently, B chromosomes arose in a single laboratory stock of *Drosophila melanogaster* that carries a mutant allele of *matrimony* and the *TM3, Sb Ser* balancer chromosome. In this genetic background, the B chromosomes exhibit biased transmission during female meiosis, a phenomenon referred to as a meiotic drive. This aberrant segregation of B chromosomes led us to wonder if their biased transmission may be more severe in other contexts that are known to disrupt meiotic chromosome segregation. In humans, the age of a female is positively correlated with improper chromosome segregation, resulting in females of advanced maternal age having a higher frequency of aneuploid pregnancies. Aged oocytes in *D. melanogaster* females are also prone to defects during meiosis, leading us to hypothesize that aged oocytes will result in a higher transmission frequency of the B chromosomes. To determine if the drive of B chromosomes increases with age, we will compare the B chromosome transmission frequency between progeny that arise from aged oocytes and progeny that arise from young oocytes. To do this, we will collect virgin females and feed them for one day, then starve them for 13 days. During this period, no new oocytes are generated, and the oocytes from the initial round of oogenesis become fully mature and are held by the female. Upon mating, oogenesis resumes and the older oocytes are laid first, followed by the newly generated young oocytes. If the age of the oocyte affects B chromosomes transmission, we expect to see an increased transmission frequency in progeny derived from the older oocytes that is followed by a return to expected levels in progeny derived from young oocytes. As we begin to understand how age can affect the transmission of B chromosomes, we anticipate using the B chromosomes as a powerful system for evaluating other environmental factors that promote aberrant chromosome segregation during female meiosis.

854S **Acetyl-CoA Carboxylase-Mediated Lipid Metabolism Determinates Oocytes by Maintaining Proper TOR Signaling Levels** Hwei-Jan Hsu[1], Oyundari Amartuvshin[2] [1]Institute of Cellular and Organismic Biology, Academia Sinica, [2]Academia Sinica

Reproduction is closely tied to nutrient intake and lipid metabolism, with imbalances often leads to reproductive failure. We characterized the metabolic mechanisms mediated by Acetyl-CoA Carboxylase (ACC, a rate-limiting enzyme for fatty acid synthesis) that support oogenesis and discovered that ACC regulates nutrient-responsive TOR signaling to maintain endosomal trafficking, crucial for oocyte determination. ACC deficiency shifts metabolism toward fatty acid oxidation (FAO), fueling the TCA cycle and electron transport chain (ETC), which hyperactivates TOR signaling. This results in excessive protein synthesis, disrupting endosomal trafficking and impairing germ cell differentiation. Restoring balance through FAO or TOR inhibition, reducing protein synthesis, or adjusting dietary protein intake corrects these defects. Our findings reveal a critical link between lipid metabolism and nutrient-sensing pathways in oogenesis, offering potential therapeutic strategies for metabolic disorders affecting reproduction.

855S **A de novo gene functioning in spermatogenesis** Bing-Jun Wang, Li Zhao The Rockefeller University

Many new genes tend to be expressed in the testis, suggesting that testis is a hotspot for genetic innovation. Among the novelties that arise during spermatogenesis, one of the most striking is the de novo birth by which functionally important genes can be born from ancestrally noncoding sequences. More and more cases of de novo gene originations have been reported, most showing testis-biased expression. However, the exact functions of these de novo genes and how they integrate into functional networks remain elusive. To investigate their roles, we identified a young de novo gene that recently emerged in the D. melanogaster species complex, showing substantial sequence divergence between D. melanogaster, D. simulans, D. sechellia, and D. mauritiana in a short evolutionary timeframe. The gene contains multiple highly similar repetitive sequences, with both sequence units and copy numbers strongly diverged among the four species. Copy number is also polymorphic among D. melanogaster strains. The gene may have evolved through the expansion of repetitive sequences, suggesting a mechanism by which newly evolved genes acquire complexity. Like many other de novo genes, this gene shows testis-specific expression, with higher expression levels in D. melanogaster and moderate expression in the other three species. The gene initiates transcription in spermatocytes, and the encoded protein is translated and functions from late spermatocytes to the canoe stage of spermatids but is absent in mature sperm. The protein localizes to the endoplasmic reticulum. We generated a knockout strain for the gene, which showed fertility defects, suggesting that the gene is functional despite its young age. These findings shed light on the evolution and function of a de novo gene in spermatogenesis, providing insights into how de novo genes can rapidly acquire functionality and complexity following their origination.

856S **Structure-guided homology detection identifies putative functional roles for proteins with domains of unknown function in the *Drosophila* male germline** Kendall Green, Talha Mazhar, Sofia Sanchez, Jeffrey Vedanayagam University of Texas at San Antonio

Nearly a fifth of the annotated protein-coding genes in well-studied model organisms, including humans, have no known function. The absence of putative function for many of these proteins can partly be attributed to the failure of homology detection at the sequence level, either due to rapid protein divergence or the potential emergence of *de novo* genes. In *Drosophila melanogaster*, 498 protein-coding genes are annotated in the PFAM database as containing 151 domains of unknown function (DUFs). Among these, 169 genes are either highly enriched or restricted to the testes based on the modENCODE tissue profiling data, yet their potential roles during spermatogenesis remain unknown. Here, we use AlphaFold protein structure predictions and the structural homology detection tool foldseek to determine the similarity of DUFs to previously characterized protein domains in PFAM.

First, to evaluate the accuracy of AlphaFold predictions, we compared AlphaFold structures for all *Drosophila* proteins for which an experimentally determined structure was available in the protein data bank (PDB) and found that the AlphaFold predictions were >90% accurate using the TM-score metric for assessing topological similarity. Next, to assess the confidence of domain similarities identified by foldseek, we examined protein structure alignments at the domain level for all previously characterized C2H2 zinc finger (n=747) and WD40 repeat (n=537) domains—two most abundant protein domains in the *Drosophila* proteome and found that foldseek accurately detected all of them with a domain alignment TM-score ≥ 0.5. We then turned to uncharacterized *D. melanogaster* DUFs and detected structural homology for 236 proteins containing 64 DUFs in the Swiss-Prot and PDB databases with a foldseek similarity e-value < 0.05.

Nearly 30% of DUF proteins for which we were able to assign a putative function based on domain similarity are highly expressed in the testes, highlighting structural-homology searches as a robust tool to discover novel spermatogenesis genes. We are currently seeking a functional understanding of two DUFs, DUF4777 and DUF4780 with putative function in post-transcriptional regulation during male gametogenesis.

Overall, our study demonstrates the effectiveness of structure-guided homology detection in investigating the putative functions of previously uncharacterized proteins. Additionally, our computational pipeline can be readily scaled to characterize DUFs in annotations from other model organisms, including vertebrates.

857S **Identifying novel components in spectrosome/fusome and their functions in *Drosophila* oogenesis** Yiming Mao[1], Allan Spradling[2] [1]Department of Biology, Johns Hopkins University, [2]Department of Embryology, Carnegie Institute for Science

Oogenesis is a fundamental process in animal kingdom. There are multiple programs during early oocyte development that are highly conserved across species, yet poorly understood, among them the regulation of fusome. In *Drosophila* germarium, fusome is a membraneous structure that can be observed from the start of mitotic division of cysts. Fusome connects the 16 cyst cells through the ring canals and played several important roles including establishing the initial polarity (The cell with more fusome content after the first division is designated oocyte,) facilitating transportation for organelles such as mitochondria from nurse cells into oocyte, and potentially playing a role in material rejuvenation. Spectrosomes are precursors for fusomes and are located on the apical side of the GSC close to stem cell niche. While previous studies have found out around 20 components within fusome and spectrosome, much remains unknown regarding the two structures' functional significance.

To further investigate the components of spectrosome/fusome, we harness the power of direct protein-trap lines generated by transposon and CRIPSR-Cas9 editing. These lines, coupled with confocal microscopy, help us efficiently screen for new genes that are housed inside the structures with sub-cellular precision. So far, we have found several genes of interest. Wdr62 and Cnx99A are two proteins that start appearing in region 2A fusome of the germarium. As Wdr62 is reported to be involved in mitotic cell cycle regulation and Cnx99A has been annotated to be acting with calreticulin, which together act as checkpoint for correct polypeptide folding within ER, both are pertinent to what we already know about fusome. Moreover, we also find out several genes associated with vesicle trafficking and lysosomal degradation which might help us better understand fusome's role in material transport and clearance. We are currently conducting RNAi and cloning experiments on those genes and hope to reveal their significance in germarium's structural integrity and oogenesis in general.

858S **The *hsrω* transcripts regulate ovulation through TBPH in *Drosophila*** RIMA SAHA[1], Subhash C Lakhotia[2] [1]Genetics, West Virginia State University, [2]Department of Zoology, Banaras Hindu University

LncRNAs and hnRNPs have been reported to function in *Drosophila* oogenesis since long back but none of them have been associated with ovulation so far. Low fecundity due to loss of *hsrω* transcripts, encoded by a developmentally active and stress-inducible lncRNA gene *hsrω*, was reported but the reasons were not identified. Here, we report that *hsrω* transcripts are required especially in follicle cells throughout oogenesis and during ovulation. Modulation of *hsrω* transcript levels varyingly perturbs oogenesis, resulting in poor ovarian growth, developmental delay, high apoptosis, and ovulation block majorly. Sudden reduction of *hsrω* transcripts is sufficient for apoptosis during mid-oogenesis. Expression of *hsrω-RH* transcript in *hsrω* near-null background rescues ovulation block along with other ovarian defects, while the cytoplasmic *hsrω-RA* transcript showed mild rescue confirming *hsrω* mis-function to underlie these defects and also characterizing *hsrω-RH* transcript's function for the first time in oogenesis. We demonstrate that *hsrω* acts in-concert with *cut* during egg chamber development and ovulation. We also identify TBPH/TDP-43, a *hsrω*-interacting hnRNP and a component of *ω*-speckle, as a modifier of ovulation. Our work establishes the essentiality of *hsrω* transcripts especially *hsrω-RH* in egg chamber development, ovulation, and therefore in fecundity along with novel functions of TBPH in follicle cells during oogenesis.

859S **The roles of PGD_2 and its synthase GstS1 in *Drosophila* eggshell development** Jie Li, Tina L Tootle Biology, University of Iowa

The *Drosophila* eggshell is a multilayered proteinaceous structure that covers the oocyte, acts as a barrier to prevent dehydration and protects the egg from toxic macromolecules. Eggshell development is regulated by multiple signaling pathways. One such pathway is prostaglandin (PG) signaling. PGs are lipids derived from arachidonic acid and exist in different bioactive forms, including PGE_2, $PGF_{2\alpha}$, PGD_2, PGI_2, and TXA_2. These bioactive PGs are each synthesized by a series of enzymes, including cyclooxygenase (COX) enzymes and type-specific synthases, and activate PG-type specific G protein-coupled receptors (GPCRs) and downstream signaling cascades. Prior work established that loss of the *Drosophila* COX-like enzyme, Pxt/dCOX1, results in defective eggshell structure and function. Further, data supports that one way PGs regulate eggshell formation is by controlling the temporal order of expression of the eggshell genes, a process critical for the proper formation of the eggshell layers. However, which specific PG mediates eggshell formation, and the mechanisms of PG action remain unclear. Here we provide evidence that loss of *Drosophila* GstS1, a putative PGD_2 synthase, greatly impairs female fertility and phenocopies the eggshell defects observed when all PG synthesis is lost. These findings suggest that PGD_2 signaling plays an indispensable role in *Drosophila*eggshell formation and female fertility.

860S **Bourbon and Mycbp function with Otu to promote Sxl protein expression in the Drosophila female germline** Meera D Gangasani, Marianne Mercer Molecular Biology, UTSouthwestern

In Drosophila ovaries, germ cells differentiate through several stages of cyst development before entering meiosis. This early differentiation program depends on both the stepwise deployment of specific regulatory mechanisms and on maintenance of germline sexual identity. The study of female sterile mutations that result in formation of germ cell tumors has been invaluable in identifying the molecular mechanisms that control these developmental events. Here, we characterize the germ cell enriched gene CG14545 that we named bourbon (bbn), null mutants of which cause the formation of a mixture of agametic ovarioles and cystic germ cell tumors. To better understand the function of Bbn, we performed proteomic analysis and found Bbn forms a complex with Ovarian tumor (Otu), a protein previously linked with regulation of the sex determination factor Sex lethal (Sxl), and the Drosophila ortholog of c-Myc binding protein (Mycbp). Loss of Mycbp results in the formation of cystic germ cell tumors, mimicking the differentiation defects observed in Sxl, otu, and bbn mutants. Bbn promotes the stability of Otu and fosters interactions between Otu and Mycbp. We find that germ cells from bbn and Mycbp mutants display a loss of Sxl expression specifically in the germline. Strikingly, transgenic rescue experiments show that the bbn sterile phenotype is independent from Sxl splicing defects. Further evidence suggests that Otu physically interacts with and promotes Sxl protein stability. This function does not depend on Otu's deubiquitinase activity. Lastly, we find that the human orthologs of Otu and Mycbp, OTUD4 and MYCBP, also physically interact, suggesting conservation of function. Together these data provide new insights into how a conserved complex promotes the germline expression of Sxl protein and the differentiation of Drosophila germ cells.

861T **Candidate based screen to identify upstream G protein coupled receptors tasked with mediating Ca^{2+} influx in the disseminating Ras^{V12}-expressing tumor cells** Izabella Thomas, Nathan Ocampo, Jiae Lee Biology, California state university long beach

Dissemination of cells within a tumor is a crucial step in the process of metastasis; however, the molecular pathways involved in this process are not fully understood. From our previous experiments, we found that the intracellular calcium signaling induces the remodeling of E-cad/β-catenin subcellular localization, which is necessary for the invasiveness of Ras^{V12}-transformed cells in vivo. E-cad/b-catenin disassembles at adherens junctions and assembles at invasive protrusions, a process which is mediated by influx of calcium which promotes the actin and cortactin-rich invadopodia-like protrusions which is an essential step for dissemination. Utilizing the Ras^{V12} tumor model in the *Drosophila* midgut, we sought to identify the upstream G-protein coupled receptors (GPCR) that regulate the calcium influx in a Ras^{V12} expressing tumor cells. This study is performed using a candidate based approach of G-protein coupled receptors, namely, mthl-5, mthl-15, Tre-1, and moody. Utilizing RNAi lines to knock down these target gene expressions in the Ras^{V12}-expressing tumor cells, we observed the inhibition of dissemination or lack thereof within dissected midgut regions. From this screen, we have identified the mthl-5 and mthl-15 genes as a potential upstream GPCR regulating tumor cell dissemination. Moreover, we have confirmed the lack of mthl-5 and mthl-15 can recapitulate the phenotype of the tumor cells with disruption in calcium influx, blocking the dissemination. Altogether, this study can illuminate an essential molecular aspect previously unknown involved in initiating metastasis providing potential targets in future therapeutic applications.

862T **Compromising Lagging-strand DNA Polymerase alpha Enhances Fly Midgut Stem Cell Regeneration In Response to Chemical Damage** Yingshan Bi CMDB, Johns Hopkins Univerisity

The Drosophila intestinal stem cell (ISC) system in the midgut is a well-characterized model to study cell fate decisions in the somatic lineages. ISC undergoing Asymmetric Cell Division (ACD) can either maintain itself, or differentiate into Enteroblasts[1].

Research in our lab revealed that heterozygous flies with reduced level of DNA polymerase alpha (Polα) activities (polα50+/-) recapitulate stem cell-specific replication-coupled asymmetric histone H3 incorporation pattern in progenitor non-stem cells. And compromised Polα activities showed enhanced regenerative capabilities during aging in both male [2,3] and female germline, as well as during tissue regeneration in male germline and in midgut ISC system [3]. My previous work demonstrated that pola50+/- infected with a lethal bacteria[4] survive significantly longer, showing higher ISC percentage when drastic declines of individual population were detected [3].

To assess whether polα50+/- confers a regenerative advantage after tissue damage in a more controlled manner, I used a well characterized chemical (Dextran Sulfate Sodium) DSS to induce the gut damage, which promotes ISC division[5]. I found that flies with fed DSS (6% or higher) exhibit gut damage and a higher temperature (29 °C) could accelerate this. Survival analysis showed that pola50+/- females survive significantly longer than control at both 6% and 9% DSS, at room temperature and 29°C.

Future experiments will assess the gut damage with smurf assay, using blue dyes added into solid food to detect if any DSS-induced gut leakage. On cellular level, ISC activity will be tracked post-DSS feeding by measuring the ISC numbers during the tissue repair through time-point immunostaining. Further work will include analyzing the ISC mitotic activities, tissue morphologies and other cellular features in pola50+/- midguts. Genome-wide analyses like snRNA-seq will be performed to investigate the differential gene expression across different cell types post DSS-feeding.

In summary, this study reveals a novel role for DNA replication components, in enhancing regenerative capabilities responding to tissue damage or aging, providing insights into the non-traditional roles of DNA replication components in development, homeostasis and tissue regeneration.

863T **Elucidating the role of multiple feedback loops in regulating germline stem cell decisions** Razeen Shaikh, Gregory T Reeves Chemical Engineering, Texas A&M University

Stem cells determine the reproductive health of a tissue and can help diagnose and treat diseases, including infertility and ovarian cancer. However, the exact mechanisms that dictate stem cells decisions (differentiation vs. self-renewal) is unknown, as these decisions are often regulated by complex and intertwined signaling networks. To address this knowledge gap, we focused on the *Drosophila* ovarian germline, which is a well-characterized model system compatible with long-term, quantitative live imaging, allowing us to visualize stem cell behavior in its native environment. The asymmetric division of the Germline Stem Cells (GSCs) results in two daughter cells–a self-renewed GSC and a differentiated Cystoblast (CB). The highly conserved Bone Morphogenetic Protein (BMP) pathway ensures self-renewal and growth of the GSCs but is downregulated in the CBs to allow for differentiation. BMP signal transduction upregulates *dad* and represses Fused (*fu*), both of which are negative regulators of the BMP pathway. Moreover, these regulatory mechanisms operate on a system of two cells which remain connected during a portion of the cell cycle. To understand the design principles which enable the BMP pathway to regulate GSCs, we developed a biologically-informed mathematical model of multi-compartment GSC division to investigate the dynamic roles Dad and Fused play in determining cell fate. Our simulations suggested that Dad optimally controls the BMP signal transduction to enable GSC homeostasis and differentiation. In dad^{KO} mutants, GSCs were more likely to divide symmetrically. To validate the model predictions, we used CRISPR-Cas9 to delete the *dad* locus, breaking the negative feedback loop. We also inserted MS2 coat-protein (MCP) binding sites into the loci of BMP pathway components such as *dad* and *fu*, to facilitate quantitative live imaging of nascent transcriptional bursts. To summarize, through the synergistic application of predictive modeling, genome engineering, and quantitative imaging we endeavor to develop a systems-level understanding of the ovarian germline stem cell division in its native environment.

864T **ABC Transporter genes are required for maintenance of the *Drosophila* male germline stem cells** Judy Leatherman, Sarah Dankwah, Trey Daulton, Brecken Lusk, Swagata Maity, Rey Ramos, Cheyenne Smith, Israel Wipf Biological Sciences, University of Northern Colorado

ATP binding cassette (ABC) transporters are a large, conserved gene family of transmembrane proteins that pump various substrates into and out of cells. They are best known for their ability to confer multidrug resistance to cancer cells due to their efflux of chemotherapy drugs, and this trait is typically associated with rare cancer stem cells that become enriched over the course of treatments. ABC transporters also confer the “side population” trait to cells, a historical way of identifying stem cell populations due to their efflux of vital dyes like Hoechst. Our lab studies the *Drosophila* testis stem cell niche, and we wondered whether the cells of this tissue might also express ABC transporters and use them to protect the stem cell populations against toxins. There are 56 members of the ABC transporter gene family in *Drosophila.* We first identified which family members were expressed in the testis niche by screening enhancer and gene trap lines, and mining the FCA single-cell RNA-sequencing data. Next, we investigated whether testis stem cells efflux the auto-fluorescent chemotherapy drug doxorubicin. We found that germline stem cells (GSCs) accumulated less drug than their differentiating daughter cells, but we were not able to establish a clear causative relationship of this trait with any single ABC transporter gene. Over the course of these experiments we discovered that multiple ABC transporters are required for maintenance of the germline lineage, unrelated to any aspects of protection against toxins. Future work is focused on screening the remaining gene family members to determine which ones function in GSC maintenance, and investigating various hypotheses for what these membrane pumps might be transporting that is critical to the maintenance of GSC.

865T **Identifying cellular and molecular mediators of germline stem cell regeneration in the *Drosophila* testis** Jasmine Grey, Erika Matunis Cell Biology, Johns Hopkins University School of Medicine

Germline stem cells (GSCs) transmit the genome to the next generation, making their survival strategies of particular interest. When GSCs are genetically ablated, their progeny (spermatogonia) undergo dedifferentiation and revert into stem cells, a conserved process rarely observed in unperturbed tissues. Spermatogonial dedifferentiation can also be induced by exogenous stressors such as irradiation or starvation. While the JAK-STAT and JNK signaling pathways have been shown to regulate spermatogonial dedifferentiation, the precise mechanisms and molecular signals governing this process remain elusive. Here, we use the *Drosophila* testis stem cell niche to investigate GSC replenishment following significant stem cell loss induced by γ-irradiation (IR). We first established conditions to monitor GSC loss and recovery after IR *in vivo*, identifying the level of IR needed to cause a 2-fold reduction in the GSC population. GSC loss was rapid, with detachment and displacement from the niche occurring within 24 hours. Remarkably, lost GSCs were replenished and their progeny returned within a week, indicating restored GSC functionality. *Ex vivo* live imaging revealed that GSC recovery post IR occurs through spermatogonial dedifferentiation and through symmetric renewals of GSCs, where both daughter cells remain attached to the niche after a GSC divides. To further uncover the cellular mechanisms underlying dedifferentiation after irradiation, we are using live imaging of testes containing marked germline and somatic lineages to observe interactions between these cell types. Preliminary live imaging data show that fragmentation of spermatogonial clusters precedes dedifferentiation, prompting us to examine germ cell connectivity upon irradiation through live imaging of the structures that interconnect the spermatogonial cysts. We are also testing candidate molecular pathways for roles in dedifferentiation using genetic and pharmacological tools. A deeper understanding of stem cell resistance to IR and the robust ability of these cells and their daughters to repopulate the niche has broader implications particularly for other radio-resistant stem cells like cancer stem cells, which contribute to the regrowth of tumors after radiotherapy.

866T **Tdrd5l promotes male identity in germline stem cells** Caitlin Pozmanter[1], Mark Van Doren[2] [1]Johns Hopkins University, [2]Mark Van Doren

Germline sex determination is regulated by a combination of signals from the somatic gonad, and germline autonomous sexual identity regulated by the RNA-binding protein Sex lethal (Sxl). Previously our lab identified *Tudor domain containing protein5-like (Tdrd5l)* as being important for male identity in the germline. Tudor-domain containing proteins are conserved across the animal kingdom for their necessary functions in germline development including post-transcriptional gene regulation. Tdrd5l is expressed in the developing germ cells and GSCs in males but is repressed in female GSCs at least in part due to regulation by Sxl. Currently we are working to understand how Tdrd5l promotes male germline identity.

Tdrd5l mutant adult testes exhibit dramatic germline loss which is often seen in mutants for sex determination factors where the sex of the germline does not match the sex of the surrounding soma. One important regulator of germline sex determination is the JAK/STAT pathway, which promotes male germline identity but is repressed in female germ cells downstream of *Sxl*. Interestingly, Tdrd5l is also important for regulating germline JAK/STAT activity. In *Tdrd5l* mutants, male GSCs showed a reduction of Stat staining indicating a loss of JAK/STAT signaling. To determine how Tdrd5l regulates JAK/STAT signaling, we screened known JAK/STAT repressors for the ability to rescue the *Tdrd5l* phenotype upon loss of function. One exciting hit from this screen is the *Drosophila* PIAS protein Su(var)2-10. Antibody staining for Su(var)2-10 in *Tdrd5l* mutant testes shows increased Su(var)2-10 expression in the germline. This suggests that Tdrd5l could promote JAK/STAT activity by repressing expression of *su(var)2-10* in the early germline.

Another important signaling pathway active in the GSC niche is the BMP pathway. Our work shows that BMP signaling (pMAD) in GSCs is sexually dimorphic and is substantially higher in female GSCs compared to male GSCs. Loss of *Tdrd5l* in the male germline results in increased pMAD expression in male GSCs suggesting possible feminization of these cells. Further, expression of Tdrd5l in the female germline led to a decrease in GSC pMAD levels comparable to male GSC pMAD levels. Thus, these data support a model where *Sxl* and *Tdrd5l* act autonomously in the germline to regulate key signals from the somatic gonad acting through both the JAK/STAT and BMP pathways to control germline sexual identity.

867T **Arginine kinase, a regulator of energy metabolism, controls the growth of the flight muscles** Maria Paula Zappia, Anton Westacott, Hannah Cooke, Rhianna Geary, Maxim Frolov Biochemistry and Molecular Genetics, University of Illinois at Chicago

Skeletal muscle contraction is a high energy demanding process that requires a significant amount of ATP. We recently built a single cell atlas of the adult muscle precursors (AMPs) and identified arginine kinase (Argk1) as a top marker for a sub-type of AMPs. Argk1 belongs to the phosphagen kinases family, which also includes the creatine kinase in mammals. The phosphagen kinases are important in maintaining ATP homeostasis during muscle contraction. While dysfunctional creatine kinase has been associated with myopathies, the role of these kinases during muscle development is not well understood.

To explore the relevance of Argk1 in muscle development, we depleted its expression using a muscle specific Mef2-Gal4 driver, which resulted in a severe reduction in ATP content and in animal lethality. Thus, data indicate that Argk1 is functionally important in muscle development by controlling cellular ATP levels and energy metabolism. Additionally, both transverse and sagittal cross-sections of the indirect flight muscles showed that the loss of Argk1 led to reduced muscle size and abnormal myofibrillogenesis, thus supporting that Argk1 controls myofibril assembly and muscle growth. Inactivating Argk1 with a late muscle driver Act88F-GAL4 also resulted in reduced muscle size, thus confirming its role as a regulator of muscle growth in late stage of myogenesis. In addition, Argk1 is required earlier in proliferating AMPs to maintain their size and proper transition through cell states. We showed by fluorescent activating cell sorting (FACS) that Argk1-depleted AMPs were significantly smaller than wild type, which resulted in strong reduction of the size of the adepithelial layers of wing imaginal discs. Moreover, we performed single cell RNA-sequencing experiments and identified defects in the progression from progenitor to differentiated state upon Argk1 depletion.

In conclusion, our results reveal a dual role for Argk1 in regulating energy metabolism during muscle development, one affecting AMPs size and state transitions prior to myoblast fusion, and another one affecting muscle growth after myoblast fusion.

868T **Assessment of heterogeneity within the *Drosophila* germline stem cell niche** Jennifer Viveiros, Erika Matunis Cell Biolgoy, Johns Hopkins School of Medicine

Adult stem cells reside in dynamic, supportive microenvironments termed niches, which are generated by specialized niche cells. Niches are often complex and composed of functionally cooperative subpopulations of cells rather than uniform populations. Gaining insight into niche composition is fundamental to our understanding of tissue homeostasis. Using the *Drosophila melanogaster* testis stem cell niche as a model, we can further our understanding of stem cell niches by investigating their cellular composition and gene expression programs. This niche contains three cell populations: post-mitotic somatic hub cells (or niche cells), which are surrounded by two types of stem cells, germline stem cells (GSCs) that differentiate into sperm, and cyst stem cells (CySCs) that give rise to somatic support cells. Previous *in situ* hybridization 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. Here, we examine hub cell heterogeneity with respect to origin, position, and function within the hub. Intriguingly, hub cells descend from somatic gonadal precursors (SGPs) that arise from three distinct parasegments (PS) in embryogenesis, suggesting that developmental origin could underlie previously observed heterogeneity. However, using lineage tracing tools, we find that adult hub cells arising from PS 11 do not differ in position or endogenous signaling compared to hub cells from other parasegments. Using a clonal marking approach, we also observe that expression of proteins integral to anchoring the hub to the tissue's apex is not required in every hub cell, but loss of these proteins from all hub cells results in hub displacement. This is supported by our results suggesting most but not all hub cells contact the testis wall. However, we do observe that hub cells losing quiescence after CySC ablation are significantly more likely to be contacting the testis wall. Future work will continue to examine the role of PS 11 cells in the adult hub and investigate differences in gene expression amongst hub cells. These findings will help determine if hub cells are functionally distinct and whether developmental origin correlates with function, localization within the niche, and transcriptional program.

869T **ESCRTs mediate Notch signaling in the testis stem cell niche** Mara R Grace, Erika L Matunis Cell Biology, Johns Hopkins University

Stem cell niches are dynamic microenvironments that provide signals to ensure the maintenance and self-renewal of adult stem cell populations. Proper signaling dynamics within the niche are crucial to maintain homeostasis, while disruption of this signaling can lead to tissue death or overgrowth. Although much is known about signaling from niche cells to stem cells, little is known about signaling in the opposite direction, from stem cells back to their niche. The testis stem cell niche of *Drosophila melanogaster* is an excellent model to investigate such signaling, specifically that of somatic stem cells, the cell population that supports the germline, to niche cells. Endocytosis regulates a myriad of signaling pathways as well as cellular communication. The ESCRT complexes are involved in a variety of cellular processes, such as multivesicular body formation and particle budding, and are considered endocytic tumor suppressor genes due to their role in signal attenuation. Here, I show that knockdown of several different ESCRT members in somatic stem cells causes the niche to become enlarged, both in cell number and overall volume, thus suggesting that ESCRTs mediate signaling from somatic stem cells back to their niche to prevent niche hypertrophy. Additionally, niche cells, which are normally quiescent, enter the cell cycle. While investigating signaling pathways that may be regulated by ESCRTs, I found that Notch activity is increased in somatic stem cells upon ESCRT knockdown. Furthermore, I have found that knockdown of Notch in somatic stem cells prevents niche overgrowth whereas expression of activated Notch in somatic stem cells results in an enlarged niche, thus suggesting that Notch signaling mediates the observed niche hypertrophy caused by ESCRT loss. This suggests a model where loss of ESCRTs in somatic stem cells leads to an autonomous increase in Notch pathway activity which then non-autonomously triggers niche overgrowth. This work demonstrates an instance of stem cell to niche communication, and, as the previously quiescent niche cells are now entering the cell cycle, has implications for tumorigenesis.

870F **Somatic ring canals contribute to the regulation of proliferation and differentiation in the ovarian follicle stem cell lineage** Michael Baumgartner[1], Andreana Gomez[2], Cristy Mendoza[2], Todd Nystul[3] [1]University of Pennsylvania, [2]UCSF, [3]UC San Francisco

In adult stem cell lineages, proliferation and differentiation must be tightly regulated so that new cells are specified in the correct time and place. This process is made more robust through the integration of signal transduction cascades with multiple cell biological inputs such as metabolic state and mechanical forces. In some cases, cells can go through an alternative form of mitosis in which ingression of the cytokinetic furrow arrests prior to abscission, leaving the sister cells connected by stable intercellular bridges called ring canals. These structures have been well studied in the male and female germline, where they are known to facilitate the intercellular transfer of cytoplasmic contents, and have also been identified in some somatic tissues, including the ovarian follicle epithelium. Building off of these prior studies, we are investigating whether the formation of ring canals functions as a cell biological input that contributes to the regulation of proliferation and differentiation in the early follicle stem cell lineage. Consistent with recent studies of germ cell ring canals, our findings suggest that follicle cell ring canals form through a stepwise remodeling of midbody proteins, Pavarotti and Peanut, into a ring structure followed by the addition of a mucin-like protein, Visgun. To further investigate this stepwise process, we developed a computational tool for segmenting 3D confocal images of the follicle epithelium and quantified the number and position of each type of ring canal structure at different stages of the follicle cell lineage. Whereas previous studies have found that over 99% of mature “main body” follicle cells have at least one Pavarotti+ ring canal (and most have more), we find that there is much less than one Pavarotti+ ring canal per cell at the early stages of the follicle stem cell lineage. We also find that the composition of ring canals is more heterogenous at these early stages. In addition, we identified several genes that regulate ring canal proteins, proliferation, and differentiation in the early follicle cell lineage. Taken together, our working hypothesis, which we are continuing to investigate, is that the formation of ring canals biases cells in the early follicle stem cell lineage toward the proliferative main body follicle cell fate whereas loss of ring canals is required for differentiation toward the post-mitotic “stalk” and “polar” cell fates.

871F **Detailing the functions of Cytokine/JAK/STAT signaling during Drosophila midgut regeneration** Xiaoyu Kang[1], Hanna Landguth[2], Bruce Edgar[1] [1]Huntsman Cancer Institute, [2]University of Utah

Understanding cytokine/JAK/STAT signaling is crucial, as it plays central roles in immune and inflammatory responses, regeneration, wound healing, and human diseases such as auto-immune disorders, inflammatory bowel diseases (IBD), and cancers. While the human inflammatory response is intricate, involving numerous cell types and cytokines, the *Drosophila* system offers a simpler model, with only three cytokines, Unpaired 1, 2, 3 and a single gene encoding their receptor, Domeless (Dome). It also has only one Janus Kinase (JAK; Hopscotch (hop)), and one known STAT transcription factor, STAT92E. Previous studies showed that the JAK-STAT pathway has important functions in *Drosophila* midgut regeneration, including cell proliferation and differentiation. However, it is still unknown what specific roles cytokine signaling plays in the different gut cell types. To address this, we first mapped the expression patterns of the different signaling components in the different midgut cell types through the regeneration process, using multiplexed Fluorescence In Situ Hybridization (FISH) and tagged proteins. We found that upd1 is only expressed in intestinal stem cells (ISCs), upd2 is expressed in both ISCs and enteroblasts (EBs), while upd3 is expressed only in enterocytes (ECs). Other JAK-STAT signaling components, including Dome, hop and STAT92E, are expressed in all cell types. However, from our study, only small cells (ISC, EB and EE) have STAT activity, as assayed using a transcriptional reporter, 10XSTAT-GFP. Following this mapping survey, our future studies will investigate JAK-STAT functions in the different midgut cell types and how these interact with the other signaling pathways, e.g., the EGFR pathway. We believe our findings will offer a valuable model for epithelial regeneration with broader implications. For example, understanding the mechanism of how IBD promotes tumorigenesis.

872F **Transcriptional co-conspirators: Tai and Yki cooperate in intestinal homeostasis** Victoria Placentra[1], Chloe Wells[1], Shilpi Verghese[2], Kenneth Moberg[1] [1]Cell Biology, Emory University, [2]Emory University

As organisms age, the proliferative capacity of stem cells declines, which in turn impairs tissue renewal and can lead to organ dysfunction and disease. How local and systemic cues precisely regulate stem cell-based tissue renewal remains unclear, particularly in the rapidly cycling intestinal epithelium. A pair of transcriptional coactivator proteins – the Hippo pathway protein Yorkie (Yki) and the Ecdysone nuclear receptor (EcR) interactor Taiman (Tai) – are each implicated in the promotion of intestinal stem cell (ISC) renewal. We previously determined that a physical interaction between Tai and Yki, mediated via respective PPxY (proline-proline-x-tyrosine) motifs and WW (tryptophan-tryptophan) domains, allows these factors to cooperatively induce target genes associated with regenerative growth of disc epithelia (e.g., *Ilp8*). As Tai and Yki each drive ISC proliferation in the adult *Drosophila* midgut, we sought to test the hypothesis that Tai:Yki binding is required for this gut renewal function, and that this interaction allows the Hippo and EcR pathways to cooperatively regulate gut homeostasis via a set of as yet undefined target genes in midgut cells, e.g., absorptive enterocytes (ECs) and ISCs. To address this question, we have generated a CRISPR mutant fly stock in which the PPxY motifs of endogenous Tai are converted to PPxA, blocking Tai's binding to Yki and allowing precise and selective decoupling of the Tai/EcR and Yki/Hippo pathways *in vivo*. Here we show these tai^{PPxA} homozygous animals are viable, sensitive to gut damage, and have reduced ISC pools in the adult midgut, which is predicted to dramatically lower their regenerative potential. At present, we are mapping the source of this tai^{PPxA} effect to ISCs or to ECs that transmit damage signals to induce ISC asymmetric divisions. Ongoing lineage tracing experiments will also assess the renewal capacity of tai^{PPxA} ISCs. Our preliminary analysis of the effect of tai^{PPxA} on the gut transcriptome detects a dramatic upregulation of the Tai-Yki coregulated gene *piwi*, which is an ISC-specific gene required for gut renewal. *piwi* enhancer trap lines will be used to map this increase to specific cell types in the adult midgut. Collectively, we hypothesize that the Tai-Yki axis may have a dual role in ISCs: to repress renewal genes such as *piwi* in the absence of gut damage, but then switch to activation of these genes in the presence of damage signals (e.g., upd3).

873F **Ionization Radiation-Induced Cell Fate Change and Translocation in *Drosophila melanogaster* Wing Disc** Michael Shiferaw Molecular, Cellular & Developmental Biology, University of Colorado, Boulder

Ionizing radiation, while a potent weapon against cancer, appears to induce a unique adaptability in cells, enabling them to survive and relocate within tissues. This phenomenon is particularly evident in the *Drosophila melanogaster* wing imaginal disc, where radiation exposure causes hinge cells to translocate unidirectionally into damaged pouch regions and change fate to replace lost cells. Given that approximately 50% of cancer patients receive radiation therapy, understanding this radiation induced cellular plasticity holds significant clinical relevance. To identify the molecular mechanisms driving this response, comparative analysis of bulk RNA sequencing data from hinge versus non-hinge populations in a time course after irradiation (Ledru et al., 2022 PMID: 34990447), along with single-cell transcriptomics data from (Worley et al., 2022 PMID: 35820420), was used to identify genes potentially driving this radiation-induced plasticity. These analyses found upregulation of transcripts that encode matrix metalloproteinase-1 (MMP1) and the serine protease homolog Scarface (scaf) in hinge cells post-radiation, which was confirmed by single molecule RNA Fluorescent In-Situ Hybridization (smRNA FISH). Ongoing functional analyses using RNAi-based knockdown of these genes specifically in the hinge aim to clarify their roles in radiation-induced cell translocation and fate plasticity.

874F **Characterization of regenerative response in Drosophila limb activated by nutrient signals** Yutian Li, Jesus M. del Rio Salgado, Lea Goentoro BBE, California Institute of Technology

In multiple species including in Drosophila, when specific nutrient signals were given to them, the animals begin to regenerate appendage or limb. This observation raises the hypothesis that the nutrient environment influences how animals respond to injury, including activating regeneration responses that do not normally occur. Building on this previous work, this study characterizes the activation of regenerative response in Drosophila limb. Activation of regenerative response can be detected as early as within a day after amputation, in the way the wound heals without melanization, suggesting alteration in inflammatory response. Subsequently, while nuclear and muscle signals normally degenerate by two weeks in the control limbs, nuclear and muscle signals are retained in the regenerating limbs. Further, regenerating limbs show modulation in the reporters of transcription factor Mef2 and Notch signaling. Finally, regrowth is preceded by a dome-shaped protrusion emerging from the wound site. The molecular and morphological characterization presented by this study provides a starting point to investigate the mechanisms of regeneration induction in adult fly limb.

875F **Genetic Screen to Identify Metabolic Factors Involved in Regulating Germline Stem Cells Development** Dayeong Yoon Division of Life Science, Hong Kong University of Science and Technology

Stem cells are unique cells that have a remarkable ability to differentiate into several types of specialized cells in the body. They can regenerate and repair damaged tissues, which makes them useful in medical research and therapies. Therefore, the primary objective of this study was to identify the specific gene involved in regulating the development of stem cells by affecting metabolic factors. Drosophila ovarian germline stem cells serve as a perfect in vivo model to understand the function of proteins in stem cells, which eventually develop into eggs. Thus, the study concentrates on these critical elements: niche cells, germline stem cells and Cytoblasts. A genetic screening approach utilizing Gal4/UAS-driven RNAi knockdown was employed in Drosophila. 50 genes of interest were targeted for knockdown using three Gal4 lines: *Nos-Gal4*, *C587-Gal4*, and *Bab1-Gal4*. These tissue-specific Gal4 lines that manipulate gene function in GSCs are used to investigate the influence on the expression levels of genes of interest in GSCs, differentiated germ cells, and other cells within the niche. It revealed the noteworthy discovery of 10 key genes, including the *sister of tout-velu, sulfateless, brother of tout-velu, CG8646, gigas, Tsc1, Akt kinase, HMG Coenzyme A synthase, vaha* and *CG7367* with significant impacts on stem cell regulation (severe loss of GSCs or accumulation of cytoblast). Further investigation into the interplay among these genes to confirm they are the key regulator of the GSCs. In addition, the study delves into the relationship between *gigas* and *Tsc1*, known to encode a tumor suppressor protein that forms a complex crucial for controlling cellular growth by antagonizing insulin and TOR signaling pathways. Understanding the roles of these genes will provide valuable insights into the molecular mechanisms that govern stem cell behavior and differentiation.

876F **LncRNA-mediated regulatory axis of Lysine-specific demethylase 1 impacts germline stem cell differentiation during fly oogenesis** Tzu-Ling Shao, Ruei-Teng Ting, Ming-Chia Lee Department of Life Sciences and Institute of Genome Sciences, National Yang Ming Chiao Tung University

Cellular differentiation is precisely regulated to support tissue development and homeostasis. Lysine-specific demethylase 1 (Lsd1), an epigenetic eraser, maintains plastic chromatin states to promote cell proliferation, while aberrant Lsd1 expression contributes to tumorigenesis. In cancer cells, associations between long non-coding RNAs (lncRNAs) and Lsd1 have been shown to influence tumorigenic gene expression, although the physiological roles of lncRNA-mediated modulations of Lsd1 remain elusive. Our recent identification of *Lsd1-Interacting Non-coding RNAs* (*LINRs*) from fly ovaries provides a unique opportunity to delineate how *LINRs* and Lsd1 function coordinately to impact cell differentiation*in vivo*. We discovered that *LINR-1* and *LINR-2* regulate fly oogenesis, at least partially, by affecting germline stem cell (GSC) expansion. In germ cells, the presence of *LINRs* is required for targeting the Lsd1-complex to specific gene loci, thereby fine-tuning the GSC's responsiveness to niche signals. Moreover, *LINR-2* was also found to affect GSC expansion in a non-cell-autonomous manner, functioning within escort cells to ensure a well-defined and narrow GSC niche. The cell-specific roles of *LINR-1* and *LINR-2* in regulating GSC differentiation reveal a novel yet intricate regulatory axis of Lsd1 mediated by lncRNAs that underlies programmed differentiation of stem cells *in vivo*.

877F **Screening Cell Adhesion Molecules for Roles in Dendrite Regeneration** Mia A Brantley[1], Jada Mars[2], Annie Danh[1], Avantika Pandiyan[1], Katherine L Thomspon-Peer[1] [1]University of California, Irvine, [2]North Carolina Agricultural and Technical State University

Dendrites can be injured by stroke, traumatic brain injury, and neonatal hypoxia. Dendrite regeneration after injury is different from typical dendrite development, and the mechanism by which dendrites respond to injury and repair themselves has not been thoroughly elaborated. In particular, the role that surrounding tissues and non-cell autonomous signals have on dendrite regeneration is unknown. To address this gap in knowledge, we utilized the dendritic arborization (da) PNS neurons of Drosophila melanogaster as a model to study dendrite regeneration, and performed a screen using RNA Interference (RNAi) to downregulate 21 different genes that encode a variety of cell adhesion molecules (CAMs). We selected cell adhesion molecules with known roles in dendrite development, and sought to evaluate their roles in regeneration. To assess how neurons regenerate after dendrite injury, we injured dendrites using two distinct methods: highly precise targeted injury using a two-photon (2p) laser, or holistic tissue injury using pinching the larval cuticle and epidermis. We tested all 21 genes for roles in the response to both precise and broad tissue injury. From this screen, we found three genes whose RNAi knockdown altered regeneration after both kinds of injury: a beta integrin subunit, inflated (*if*); a cell-surface receptor, Semaphorin 2A (*Sema2A*); and a well-known morphogen, slit (*sli*). RNAi against *if* reduced regeneration following 2p laser injury and elicited a degenerative phenotype following the pinching injury. RNAi against *Sema2A* increased branch number in the regenerated arbors following both forms of dendrite injury. Following the 2p laser injury, RNAi against *sli* increased the length of regenerated branches, while the invasive capacity of existing branches increased following the pinching injury. We next plan to examine the roles of these genes in dendrite regeneration using full-body or cell-specific mutants. Together, this screen has identified 3 promising candidate genes with roles in non-cell-autonomous regulation of dendrite regeneration. Ultimately, our results will identify how the neuron's surrounding tissue and extracellular matrix can both support and hinder the regeneration of dendrites.

878F **Adult *D. melanogaster* show age-dependent decline in dendrite regeneration** Rostislav Brichko[1], Katherine Thompson-Peer[2] [1]Developmental and Cell Biology, University of California, Irvine, [2]Developmental & Cell Biology, University of California, Irvine

Neuronal dendrites undergo age-related structural changes that may contribute to circuit and synaptic dysfunction before neurons die. While dendrites can regenerate after injury in aging animals, regeneration becomes increasingly limited with older age. Manipulating insulin metabolism by dietary restriction can increase lifespan in a variety of animal model systems and may boost dendrite regeneration. The cellular and molecular mechanisms by which insulin metabolism affects dendrite regeneration in aging adults are unknown. We hypothesize that extending an organism's lifespan may enhance the health span of neurons to boost dendrite regeneration after injury, especially in older animals and at later stages after injury. Here, we show that older adult *Drosophila melanogaster* continue to regenerate their dendrites for weeks after injury. While regeneration in older animals is more significant than previously appreciated, in wild-type animals it does not fully recover total dendrite length and area coverage. Our work also investigates whether intervening in insulin signaling may boost dendrite regeneration in older adult *Drosophila*. Together this demonstrates an increased ability of neurons to regenerate after injury in older animals, and a potential avenue to further boost regrowth after trauma.

879F **The self-repressive zinc finger transcription factor Chronophage regulates intestinal stem cell proliferation and differentiation** Siamak Redhai[1], Nick Hirschmüller[2], Tianyu Wang[1], Shivohum Bahuguna[1], Svenja Leible[1], Stefan Peidli[2], Erica Valentani[1], Sviatoslav Kharuk[2], Michaela Holzem[1], Lea Bräckow[1], Fillip Port[1], David Ibberson[3], Wolfgang Huber[2], Michael Boutros[1] [1]DKFZ, [2]EMBL, [3]Heidelberg University

Intestinal stem cells (ISCs) generate various cell types crucial for homeostasis, including Enteroendocrine cells (EEs), which have important roles in physiological processes by secreting hormones systemically. Notch signalling regulates ISC fate, favouring enterocyte commitment when Notch activity is high and EEs when activity is low. However, the temporal dynamics of transcription factors that drive EE lineages remain unclear. Here, we find that *scute*, a Achaete-Scute Complex (AS-C) protein, binds to the genomic locus of the poorly characterised zinc finger transcription factor *Chronophage* (*Cph*) to promote its expression early along the ISC-to-EE lineage when *Notch* is deactivated. Our genetic and single-cell RNA sequencing experiments demonstrate that *Cph* reprograms the transcriptome of ISCs and maintains EEs across different intestinal regions. Using cell type specific genome-wide profiling of Cph binding sites, we show that Cph directly regulates the expression of key genes involved in proliferation and differentiation, while simultaneously repressing its own expression. This inhibitory feedback loop safeguards ISCs from undergoing autophagy-dependent cell death, ensuring differentiation is faithfully executed. Our findings mechanistically demonstrate how *Cph* sustains intestinal epithelial homeostasis and could represent a conserved strategy for balancing proliferation and differentiation in other tissues and species.

880S **Coordination of cell signaling during the cellular immune response in the *Drosophila* lymph gland** Xinwen Zhu, Guy Tanentzapf Department of Cellular and Physiological Sciences, University of British Columbia

Stem cell fate and behavior are governed by signals derived from their local microenvironment, also known as the stem cell niche. An important model for interactions between stem cells and their niche is the process of hematopoiesis, the production of new blood cells from hematopoietic stem cells. In *Drosophila* larvae, the main site of hematopoiesis is the primary lobe of the lymph gland. The primary lobe contains three distinct zones: the posterior signaling center houses the stem cell niche, the medullary zone houses the blood progenitors, and the cortical zone contains the differentiated blood cells. The niche provides a nourishing environment and acts as a source for signals that are essential for maintaining the balance between stem cells and differentiated blood cells.

Previous efforts in our group have established a novel system for *ex vivo* long-term quantitative imaging of the lymph gland. This live imaging platform has revealed the existence of a gap junction-mediated calcium signaling network that coordinates cell fate decisions in blood progenitors and in the hematopoietic niche. As the primary role of blood cells in *Drosophila* is to participate in immune functions, hematopoiesis is regulated by systemic cues such as infection. We hypothesize that the calcium signaling network is responsive to systemic signals and ensures a robust, coordinated downstream response in the hematopoietic stem cells of the primary lymph gland. Here, we report our findings on cell signaling in the lymph gland during the immune response to infection obtained through live imaging of genetically encoded biosensors for calcium and other signaling pathways.

881S **Degradation of Mitochondrial Cyclin E is Sufficient for Entry Into Stem Cell Quiescence** Miriam Gonzaga[1,2], Sahiti Peddibhotla[1,2], Yasha Goel[1,2], Riya Keshri[1,2], Rui Xu[1], Tung C. Chan[1], Enmeng Xu[1,2], Tricia Zhang[1,2], Shelley A. Caisley[1,1], Anne-Marie Pret[3], Beatriz Estrada[4], Julie Mathieu[1,1,5], Hannele Ruohola-Baker[1,2] [1]Department of Biochemistry, University of Washington, [2]Institute of Stem Cell and Regenerative Medicine, University of Washington, [3]Institute for Integrative Biology of the Cell (I2BC), Université Versailles Saint Quentin-en-Yvelines, [4]Departamento de Biología Celular, Universidad de Sevilla and Instituto de Biomedicina de Sevilla (IBiS), [5]Department of Comparative Medicine, University of Washington

Under acute genotoxic stress, such as chemoradiation therapy, stem cells can undergo temporary cell cycle arrest at the G1/S phase transition to avoid apoptosis. This protective state, called quiescence, is reversible once stress-free conditions allow re-entry into the cell cycle to regenerate daughter cells. We have previously demonstrated a common mechanism by which two types of stem cells, Drosophila germline stem cells (GSCs) and human-induced pluripotent stem cells (hiPSCs) enter into quiescence. Recently, we have observed a reserve of Cyclin E (CycE) associated with the outer mitochondrial membrane (OMM) that is present in normal GSCs and hiPSCs but is reduced with mitochondria in quiescent stem cells. The degradation of CycE is associated with that of the mitochondria via mitophagy, mediated by Parkin E3 ubiquitin ligase activated by Ser/Thr kinase PINK1. However, the mechanism by which CycE modulates mitophagy-dependent quiescence is unclear.

We hypothesize that CycE is degraded by Parkin-dependent ubiquitination on the OMM in quiescence and test the following mechanisms:

1. To determine whether CycE degradation is sufficient for entry into quiescence in Drosophila GSCs, we overexpressed a non-degradable form of CycE (deleted of its degradation-promoting PEST domain) using the GAL4/UAS system. Upon irradiation, we found that cells overexpressing non-degradable CycE continue cell division whereas control cells undergo quiescence. We use the same system to determine whether CycE degradation is upstream or downstream of mitophagy. However, we observed that GSCs overexpressing this non-degradable CycE still undergo mitophagy post-irradiation. This suggests that CycE degradation is downstream of mitophagy and is sufficient for quiescence.

2. To investigate the biochemical mechanism of how CycE is localized to the OMM, we will conduct site-directed mutagenesis on CycE in human fibroblasts. This method will help us determine which specific regions of CycE are required for mitochondrial localization and identify CycE-binding proteins directly involved in this mechanism.

Identifying the mechanism of CycE regulation in stem cell quiescence is critical to understanding how cancer stem cells can evade apoptosis during chemotherapy to proceed tumor regrowth post-injury, thus aiding the development of anti-cancer treatments.

882S **Epithelial cell fusion is required for tissue repair following UV-A irradiation** Lillie Mitchell, Minqi Shen, Vicki P Losick, Lydia Boer Boston College

Polyploid cells are life's stress responders. Cell cycle dependent and independent mechanisms lead to the generation of mononucleated and multinucleated, polyploid cells. The more than doubling of a cell's nuclear genome by endoreplication has been found to be an adaption to genotoxic stress enabling cell survival despite DNA damage. However, it remains unknown whether cells that increase ploidy via multinucleation also provides resilience against genotoxic stress. Here we utilize the adult *Drosophila* epithelium which we found responds to stress via the generation of multinucleated, polyploid cells. Low dose UVA irradiation (25mJ) causes epithelial cells to endure permanent DNA damage and cell loss. Epithelial integrity is then restored by 7 days via cell fusion. Epithelial specific expression of a dominant negative Rac GTPase inhibits cell fusion leading to defects in epithelial cell junction remodeling at 2-3 days. At this time, we also observe an increase in apoptosis suggesting that epithelial cell fusion is necessary for tissue repair post UVA irradiation. Epithelial cells also undergo endoreplication, but knockdown of cell cycle genes, including CycE, does not affect tissue repair. In conclusion we have discovered that enhancing cell ploidy via multinucleation is another strategy to protect against genotoxic stress and enable tissue repair. Thus, our on-going studies aim to elucidate whether different ploidy states enable cell survival and tissue repair via similar or distinct molecular mechanisms in response to life's insults.

883S **Factors Required for Nuclear Pore Complex Rejuvenation during Drosophila Oogenesis** Tram Nguyen Biology, San Diego State University

The nucleus plays a critical role as a control center of a eukaryotic cell as it contains cellular genomes. The nucleus is enclosed by the nuclear envelope, separating its contents from the cytoplasm. Embedded in the envelope are nuclear pore complexes, which function as a selective barrier to regulate the entry and exit of proteins and RNAs, controlling nucleocytoplasmic transport, regulation of gene expression and cellular signaling. The nuclear pore complex (NPC) is composed of around 30 different components, called nucleoporins (NUPs), which are highly conserved across eukaryotes. Together with nuclear lamina and nuclear matrix, NUPs help maintain the shape and integrity of nuclei and organize the genetic material. Dysfunction of NPCs and mutations in NUPs are linked to neurodegenerative and cardiovascular diseases, and to cancer. Despite their importance in these aging-associated diseases, little is known about how NPCs and NUPs are maintained and turned over. Learning such mechanisms of NPC rejuvenation can help in prevention of aging-associated disease, particularly neurodegenerative conditions. Our research investigated mechanisms of NPC turn-over using the model of Drosophila oogenesis. Oogenesis has built-in mechanisms for rejuvenation of cellular components because it must generate an oocyte that will become the next generation. We and others have identified striking NPC depletion during the early stage of Drosophila oogenesis, marked with an early differentiation marker Bam. This depletion, which we detect by immunofluorescence co-staining and microscopy imaging of NPCs and Bam, is indicative of active removal of NPCs as the initial step of their turn-over. Using oogenesis-specific RNA interference, we knocked down candidate genes to determine which of them can rescue the observed NPC depletion and thus regulate NPC turn-over. Previous research has shown that the ESCRT-III/VPS4 complex contributes to NPC turn-over in yeast and in Drosophila models of ALS/FTD. In agreement with this work, we identified a specific component of ESCRT-III/VPS4, downregulation of which can rescue NPC levels. In the future, we plan to knock down additional genes that code for ESCRT-III proteins, NUPs, and other candidate factors to test their effects on NPC depletion and in this manner, decipher mechanisms that regulate NPC rejuvenation.

884S **Regulation of Intestinal Stem Cells and Longevity by the Nuclear Envelope Protein Klaroid (Koi)** Ithan Cano[1], Helia Yaleh[2], Carlos Asturias[2], Wiam Jurdi[2], Mariano Loza-Coll[2] [1]Biology, California State University Northridge, [2]California State University Northridge

Multicellular organisms use oligo-potent Adult Stem Cells (ASCs) to maintain tissue homeostasis. ASCs undergo self-renewing asymmetric divisions, by which they produce a new copy of the ASC and a differentiating sister that will replace cells lost to injury, disease or tissue turnover. In our lab, we study the genetic regulation of a specific type of ASC, the intestinal stem cells (ISCs). Here we report our preliminary findings related to the nuclear membrane protein Klaroid (Koi), an experimentally validated target of the transcription factors Esg and dSTAT92E, two important master regulators in Drosophila ISCs. While previous work by others had established Koi's role in repairing double-strand DNA breaks in cultured Drosophila cells, and its requirement for attachment of the nucleus to the cytoskeleton in the Drosophila eye, no previous studies have specifically addressed Koi's role in ISCs.

Here we used ISC-specific knock-down and overexpression of Koi, coupled with immunofluorescence microscopy, to determine the effect that Koi manipulations have on the number, morphology and relative abundance of different cell types of the intestinal epithelium, both in young and aged flies. We have also preliminarily explored the two previously reported functions of Koi in other systems, by determining how Koi manipulations affect nuclear positioning in ISC and their immediate progeny, or the amount of detectable DNA damage in these cells.

Since our observations indicated that Koi overexpression might be protective against aging-induced intestinal dysplasia, we also conducted lifespan assays. Flies with altered levels of Koi expression in ISCs showed lifespan profiles that are significantly different from that in controls, with comparable survivorship for approximately 40 days, followed by a sharp onset of mortality afterwards. Because this sudden mortality correlated with the formation of a noticeable biofilm on the fly food surface, we explored the possibility that Koi manipulations may have drastically altered the fly's intestinal microbiome over time. However, a comparisons of microbiome profiles across genotypes revealed differences in microbial composition that preceded Koi manipulations, consistent with genetic background effects. We are currently re-investigating whether Koi manipulations affect lifespan in axenic flies, to directly address these potential confounding effects.

885S **Stem cells regulate the size of the niche during stem cell loss and replacement** Ellen Ward[1,2], Hannele Ruohola-Baker[1,3] [1]Department of Biochemistry, University of Washington, [2]Institute for Stem Cell and Regenerative Medicine, School of Medicine, University of Washington, [3]Institute for Stem Cell and Regenerative Medicine, University of Washington

Adult stem cells maintain the body post-embryonic development. While these cells are essential for tissue homeostasis, many adult stem cell populations are not immortal. Rather, they are lost and replaced stochastically. Using the *Drosophila* ovarian germline stem cell niche, we show that a signal from the stem cells regulates the architecture of the niche during stem cell loss and replacement. This work addresses three questions. First: what happens to the niche cell population during stem cell loss/replacement? Significantly, we show that the niche cell population contracts prior to stem cell loss and expands prior to stem cell attachment/replacement. Second: how do stem cells regulate the adjacent niche population? Reducing *Delta* ligand in the germline stem cells (GSCs) results in a decrease in the niche cell population, prior to stem cell loss. Increasing *Delta* in the stem cells produces an expansion of the niche cells, prior to an increase in stem cells. Reducing/increasing Insulin Receptor levels within stem cells results in a corresponding decrease/increase in the niche cell population prior to stem cell loss/expansion, similar to changes observed with altering Delta signaling. These results suggest that Insulin Receptor signaling within stem cells regulates Delta, which in turn regulates the niche cell population. Third: how do Delta ligand levels regulate the niche cell population? Within germline stem cells, a unique organelle, called the spectrosome, associates with the membrane at the stem cell/niche cell boundary. At this interface, Notch receptor accumulates in the membrane of the niche cells. Normally, the spectrosome is round. However, under high Delta levels, the morphology of the organelle elongates dramatically along the cortex of the stem cell, greatly expanding the organelle's contact area with the niche. We propose that the spectrosome delivers Delta to the stem cell membrane in order to signal to Notch on the adjacent niche cells. The strength of this Delta signal from the stem cells regulates the plasticity of the niche during stem cell loss and replacement.

886S **Deciphering the regulation of the replicative DNA polymerases in Drosophila male germline stem cells** Emma Troisi, Xin Chen Department of Biology, Johns Hopkins University

In many organisms, germline stem cells (GSCs) are able to produce countless gametes and, in some cases, are able to generate new gametes throughout the organism's life. How they are able to divide in such a manner that allows both renewal of the niche as well as the production of differentiated gametes is not entirely clear. GSCs can divide to produce one self-renewed daughter stem cell and one differentiating daughter cell despite giving them identical genetic information in a process called asymmetric cell division (ACD). We use the Drosophila male germline to study the mechanism and regulation of ACD in GSCs. Previous work from our group showed that the two daughter cells that result from an ACD in GSCs receive different epigenetic information: the daughter stem cell inherits mostly parental histones that were present in the GSC chromatin before division while the spermatogonial cell (SG) inherits mostly newly synthesized histones. During S-phase in GSCs, parental histones are biased to the leading strand while new histones are biased to the lagging strand, suggest that asymmetric histone incorporation occurs during DNA replication. Furthermore, the catalytic subunits of both lagging strand enriched polymerase complexes, Polα and Polδ, are present at a lower level in GSCs as compared to SGs, while the leading strand enriched polymerase, Polε, shows comparable levels. Compromising Polα leads to local asymmetry in histone incorporation in SGs, implying its importance in histone separation. However, how GSCs specifically maintain this differential expression of Polα and Polδ, but not Polε, is unknown. I hypothesize that a GSC-specific mechanism exists to ensure low levels of lagging strand polymerases to facilitate ACD. Using multiple techniques including RNA-FISH, enhancer-reporter assays, and post-translational modification analysis, I plan to determine where in the process of protein expression the lagging strand polymerases are facing differential regulation compared to the leading strand polymerase. Furthermore, work from our group indicates that *polα*$^{+/-}$ flies have higher fertility, a longer reproductive lifespan, and more GSCs throughout aging as compared to wild type flies. However, whether *polδ*$^{+/-}$ flies also have such advantages is not known. Understanding how modulating the levels of Polδ can influence fertility, GSC dynamics, and differentiation can further help us understand why lower lagging strand polymerase levels may be advantageous. Using a genetic ablation assay to remove GSCs, I can also describe how Polδ levels can contribute to the frequency and efficacy of dedifferentiation of SGs in response to loss of GSCs— a phenomenon with relevance to aging and regenerative medicine.

887S **Effects of hypoxia-reoxygenation on intestinal homeostasis in *Drosophila*** Prajakta Bodkhe, Savraj Grewal Biochemistry and Molecular Biology, University Of Calgary

Oxygen is essential for the growth and homeostasis of tissues. However, certain disease conditions like stroke, ischemia, and pulmonary disorders, can reduce oxygen availability, causing tissue damage. Low oxygen in the intestine has been reported to cause a state of local inflammation, affecting intestinal tissue function and integrity. The adult *Drosophila* intestine shows similarities to the mammalian intestine in terms of conserved cell types and signalling pathways. Here, we use adult *Drosophila* to explore how low oxygen conditions (hypoxia) affects intestinal tissue function. Mechanisms underlying intestinal homeostasis in adult *Drosophila* have been extensively studied, both in the context of external stressors and normal wear-and-tear. However, intestinal adaptations to oxygen deprivation are relatively under explored. We find that exposing mated female flies to severe but acute systemic hypoxia (1% Oxygen for 6 hours) causes a rapid 40% reduction in their intestinal length. This is orchestrated through widespread cell shape changes (marked by Dlg, Phalloidin) and apoptosis (marked by DCP-1, TUNEL) in the differentiated cells (enterocytes) of the epithelium. However, this effect of hypoxia on intestinal morphology and length were repaired by a brief period of re-oxygenation after hypoxia exposure. We show that re-oxygenation mediates repair through inducing a wave of stem cell proliferation (marked by phospho-histone H3+ve nuclei) and differentiation to form new mature cells. Interestingly, this injury-repair mechanism was independent of the canonical regulator of hypoxic responses, Hypoxia-Inducible Factor-1 (HIF-1). Instead, we found that it was dependent on *unpaired2,3* cytokine (mammalian Interleukin-6 family homolog) signalling from the enterocytes to stimulate stem cell proliferation and differentiation through JAK/STAT activity in these cells. Blocking *unpaired2,3* production from enterocytes or JAK/STAT activity in stem cells prevented resizing of the intestine during re-oxygenation. In future studies, we will explore how cells induce cytokine signalling in hypoxia to carry out epithelial repair. Due to the conserved nature of signalling pathways implicated in intestinal repair, our work will provide insight into how intestinal epithelial cells respond to and maintain homeostasis in hypoxia.

888S **Numb provides a fail-safe mechanism for intestinal stem cell self-renewal in adult Drosophila midgut.** MENGJIE LI[1], MENGJIE LI[2] [1]UTSW, [2]Molecular Biology, UTSW

Stem cell self-renewal often relies on asymmetric fate determination governed by both niche signals and cell-intrinsic factors. In adult *Drosophila* midgut, asymmetric Notch (N) signaling inhibits intestinal stem cell (ISC) self-renewal by promoting its differentiation into enteroblast (EB) whereas epithelium-derived BMP promotes ISC self-renewal by antagonizing N pathway activity. Here we provide evidence that the N inhibitor Numb acts in parallel with BMP signaling to promote ISC self-renewal. Although Numb is asymmetrically segregated in about 80% of dividing ISCs, its activity is largely dispensable for ISC fate determination under normal homeostasis. However, Numb becomes crucial for ISC self-renewal when BMP signaling is compromised. Whereas neither Mad RNAi nor its hypomorphic mutation led to ISC loss, inactivation of Numb in these backgrounds resulted in stem cell loss due to precocious ISC-to-EB differentiation. We also observed a mild stem cell loss phenotype associated with *numb* mutant clones, which was exacerbated in response to injury. Because BMP signaling is fluctuated in adult midgut especially after injury, the asymmetrical segregation of Numb into the future ISC may provide a fail-save mechanism for ISC self-renewal, which is essential for ISC maintenance in regenerative guts.

889S **Genetic induction of copulatory wounding in fruit flies** Sophie L Jalkut[1], Daria A Perminova[1], Madisen K Caferro[2], Vicki P Losick[1] [1]Boston College, [2]Biology, Boston College

Copulatory wounding is an unusual, but not uncommon, behavior observed in the animal kingdom. Copulation injuries can occur either intra- or inter-genitally and can be induced by behavior or morphological differences. These injuries typically incur significant costs for the female, often leading to a reduction in her lifespan, while simultaneously improving male fitness. Using the *Drosophila melanogaster* model, we unexpectedly discovered that overexpression of a serine protease is sufficient to induce copulatory wounding. Notably, the wounds did not occur on virgin females but arose after a single copulation event, resulting in extensive injury to the posterior ventral abdomen. These copulatory wounds also resembled needle puncture wounds as both develop melanization at the site of injury. Further characterization revealed that this genetic induction of copulatory wounding is dependent on the enzymatic activity of the serine protease, as well as the GMR51F10-Gal4 driver, which controls gene expression in both adult ventral epithelium and certain neurons in the brain. Therefore, we are assessing whether the overexpression of the serine protease alters *Drosophila's* mating behavior or its anatomy, leading to this remarkable phenotype. Additionally, we found the copulatory wound heals similarly to a puncture wound, relying on the generation of a giant, multinucleated polyploid cell to compensate for cell loss. This suggests that wound-induced polyploidization may have evolved to repair similar injuries in nature.

890T **Micro-C: A Powerful Tool to Study 3D Genome Organization Across Diverse Fly Tissues** Xiao Li, Michael Levine Princeton University

Three-dimensional genome organization plays a crucial role in gene regulation and development. We used Micro-C, a high-resolution chromosomal mapping technology, to examine 3D genome architecture across various Drosophila tissues, focusing on differences in chromatin looping patterns and their functional relevance to gene expression. Our analysis revealed significant tissue-specific differences in chromatin loops, with distinct looping configurations aligning closely with the transcriptional activity of key developmental genes. By comparing chromatin interaction landscapes, we identified conserved loops that persist across tissues, as well as unique structures associated with tissue-specific gene regulation. These findings support a model in which tissue-specific chromatin architecture is intricately linked to gene regulatory programs, influencing developmental processes. This study underscores the utility of Micro-C in capturing detailed insights into 3D genome organization across multiple cell types, advancing our understanding of the regulatory landscape in Drosophila and shedding light on conserved principles of chromatin architecture in metazoans.

891T **Transgenic sensors reveal compartment-specific effects of aggregation-prone proteins on subcellular proteostasis during aging** Fabio Demontis St. Jude Children›s Research Hospital

Loss of proteostasis is a hallmark of aging that underlies many age-related diseases. Different cell compartments experience distinctive challenges in maintaining protein quality control, but how aging regulates subcellular proteostasis remains underexplored. Here, by targeting the misfolding-prone Fluc^{DM} luciferase to the cytoplasm, mitochondria, and nucleus, we established transgenic sensors to examine subcellular proteostasis in Drosophila. Analysis of detergent-insoluble and -soluble levels of compartment-targeted Fluc^{DM} variants indicates that thermal stress, cold shock, and pro-longevity inter-organ signaling differentially affect subcellular proteostasis during aging. Moreover, aggregation-prone proteins that cause different neurodegenerative diseases induce a diverse range of outcomes on Fluc^{DM} insolubility, suggesting that subcellular proteostasis is impaired in a disease-specific manner. Further analyses with Fluc^{DM} and mass spectrometry indicate that pathogenic tau^{V337M} produces an unexpectedly complex regulation of solubility for different Fluc^{DM} variants and protein subsets. Altogether, compartment-targeted Fluc^{DM} sensors pinpoint a diverse modulation of subcellular proteostasis by aging regulators.

892T **A multi-omic protocol to profile chromatin accessibility, whole transcriptome and proteome in fly brains** Siyuan Feng, Jamie Freeman, John Pool Department of Genetics, University of Wisconsin Madison

Multi-omic profiling of bulk tissues provides a cost-effective approach for investigating gene regulation across molecular levels. To ensure comparability, different -omic assays should ideally be conducted on the same biomaterial. However, for many systems, it is challenging to apply bulk multi-omics to a single individual due to the limited amount of material. Here, we developed a multi-omic protocol optimized for ATAC-seq, whole transcriptome RNA-seq, and proteomics using brains. This protocol is simple and budget-friendly to implement, and has the following key advantages: 1) enabling multi-omic integration by homogenizing tissue from multiple individuals and partitioning the homogenate for each -omic assay, 2) experimental parameters are optimized for fragile tissues like brains, 3) allowing flexible experimental planning with built-in safe-stops and options for cryopreservation, and 4) providing intermediate backup samples that enable the rescue of a specific -omic experiment without restarting the entire process. This protocol is particularly beneficial for researchers investigating gene regulation in the *Drosophila* nervous system, and can be adapted for similarly sized tissues.

893T **A high-throughput recording platform and data analysis pipeline for Drosophila screening** Ryan S O›Neill[1], Solomon Aviles[2], Jacie M Cheng[2], Peter Donley[3], Marcial Garmendia-Cedillos[3], Ghadi H Salem[3], Nasser M Rusan[2] [1]National Heart, Lung, and Blood Institute, NIH, [2]CDBC, National Heart, Lung, and Blood Institute, NIH, [3]IDEAS, National Institute of Biomedical imaging and Bioengineering, NIH

Historically, Drosophilists relied heavily on direct observation under a microscope to identify mutant phenotypes. Although direct observation does not lead to deep mechanistic insight, its major advantage is lack of experimental manipulation and thus minimal preparation time, both desirable qualities for screens. However, the time required for observation is the main bottleneck for taking advantage of this direct approach. We are aiming to overcome this bottleneck using automation and artificial intelligence, thus facilitating high-throughput screens using direct observation with minimal time cost. To this end, we developed a high-throughput imaging platform for recording high-resolution movies of fruit flies, and are developing a corresponding data analysis pipeline that uses artificial intelligence to classify movies at different levels. Our imaging platform has a modular design with multiple recording units consisting of a Raspberry Pi 5 controlling an IMX477 camera, which can be scaled in number depending on the user's need. Each recording unit images six flies simultaneously at 40 FPS and in full color and resolution of 65 pixels/mm. The design and build instructions for our platform will be made open source. Our initial goal is to analyze fly behavior. The first step in data pre-processing is to apply a custom DeepLabCut foundation model trained on flies with multiple different observable phenotypes, which applies 30 keypoints to each frame of the movie. These keypoints data are then passed as input into a transformers-based neural network, which can be trained to classify movies at different levels of granularity. We are currently working on improving model performance and implementing a morphology-based pipeline. Overall, our ongoing work in developing an open source imaging platform and automating screening pipelines should be of interest and prove to be a valuable resource to the broader *Drosophila* community.

894T **Characterization of shock wave effects using gold nanoparticles and DNA in syncytial embryos of *Drosophila melanogaster*** Daniel Tapia Merino, Juan Rafael Riesgo Escovar, Achim Max Loske Mehling Universidad Nacional Autonoma de Mexico

The aim of this study was to achieve insertion of exogenous material into early *Drosophila* embryos by means of shock wave-induced fluid microjets. We utilized embryos at the syncytial stage. This facilitated insertion, as fewer membranes had to be traversed. Shock waves have been reported to be effective in permeabilizing cell membranes and walls, like those of filamentous fungi. We used gold nanoparticles as fiducial markers to trace their presence in sections of *Drosophila* larval tissues by transmission electron microscopy (TEM) after exposure to underwater shock waves. Following our protocol, most embryos treated with shock waves in the nanoparticle suspension survived to adulthood. Additionally, we assessed the insertion of a GFP-encoding plasmid by applying shock waves to embryos of a fly line commonly used to test genetic transformation by microinjection; resulting larva after treatment showed an increase in fluorescence. The demonstration that exogenous material can penetrate the outer layers (chorion and vitelline membranes), as well as the cellular membrane of early *Drosophila* embryos applying this regime, suggests a promising avenue for various biological applications.

895T **Comparing methods for the enrichment of circulating exosomes from *Drosophila* hemolymph** Akimi Green, Young Kwon Biochemistry, University of Washington

Extracellular vesicles (EVs) are critical mediators of interorgan and intercellular communication during homeostasis and disease. Bioactive molecules contained within EVs, including metabolites, miRNA, enzymes, and mRNA, facilitate communication between neighboring cells and distant tissues. EVs are secreted by most cell types and exist in biological fluids including blood, saliva, semen, and breast milk. Exosomes are a subset of EVs, ranging in size from 40-160 nm, produced from endocytic vesicles. To study circulating exosomes *in vivo,* it is essential to establish a procedure for the reliable isolation of exosomes from an animal. The robust genetic toolbox of *Drosophila* is a promising platform to discover molecular mechanisms involved in basic exosome biology.

We adapted 3 of the most common exosome isolation methods to isolate exosomes from hemolymph; solvent precipitation, ultracentrifugation (UC), and size exclusion chromatography (SEC). We collected whole hemolymph from 3rd instar larvae and subject it to low-speed centrifugation to pellet cells and large vesicles. This plasma fraction is subjected to a commercial solvent precipitation kit (XENO-EVI), UC, or SEC. The resulting exosome fractions were analyzed for yield and purity. Solvent precipitation enriches for ~20nm and ~300nm particles. Considering the heterogeneous composition of hemolymph, solvent precipitation may isolate all circulating particles including lipoproteins and large EVs. UC enriches particles between 10-150nm, however cryo-TEM imaging showed substantial contamination with ~20nm sized particles. Contamination of the UC exosome pellet with lipoproteins was confirmed via immunoblot. SEC purification yields ~3 fractions enriched for particles between 60-200nm. SEC fractions are too dilute to analyze via nanoparticle tracking analysis or immunoblot. Lyophilization of exosome-containing SEC fractions and resuspension in a smaller volume successfully increased exosome concentration.

Overall, our results show that SEC followed by lyophilization yields the purest fraction of exosomes from *Drosophila* hemolymph. Next, we will compare the transcriptomic and proteomic composition of exosome fractions isolated with these 3 methods. Ultimately, we will identify a reliable protocol to isolate and characterize circulating exosomes from hemolymph, establishing *Drosophila* as a model for interrogating the biology of exosomes *in vivo*.

896T **MARRVEL and ModelMatcher: publicly available web services that facilitate collaborative research on rare diseases** Shinya Yamamoto[1,2], Seon-Young Kim[1,2], Zahid Shaik[1,2], Michael F Wangler[1,2], Hugo J Bellen[1,2], Zhandong Liu[1,2] [1]Baylor College of Medicine, [2]Texas Children's Hospital

The diagnosis and therapeutic research of rare diseases require the integration of diverse data sets and close collaboration between clinicians and scientists. To identify disease-causing genes and variants, clinicians must gather information from various databases, including previously identified disease-gene relationships, population genomic datasets, and multiple variant pathogenicity prediction algorithms, in addition to the patients' phenotype and genotype information. When an individual is suspected of having a novel genetic disorder, it becomes essential to collect additional information associated with the candidate human gene and its orthologs in model organisms such as mice, fruit flies, worms and yeast. For therapeutic research, it is crucial to effectively identify collaborative scientists with expertise in specific genes, biological pathways, or experimental paradigms. However, such information is scattered across the internet and is difficult to access comprehensively. Additionally, some valuable information, such as unpublished scientific data on poorly characterized genes, is not accessible.

To overcome these barriers, we have been developing a suite of publicly accessible bioinformatic tools. **MARRVEL** (Model organism Aggregated Resources for Rare Variant ExpLoration, https://marrvel.org/) integrates numerous human genomic and genetic databases with various model organism databases to facilitate rare disease diagnosis. **ModelMatcher** (https://www.modelmatcher.net/) is a matchmaking service designed to foster collaborations between basic scientists with expertise or interest in specific genes and clinicians, patients, caregivers, and other stakeholders.

Drosophila researchers can take advantage of these tools to increase the clinical relevance of their findings in the laboratory and proactively engage in translational research that have direct clinical impact.

897T **Expanding the Fourth Chromosome Resource project: CRISPR-induced mutations for clonal analysis of fourth chromosome genes** Brandon P Weasner[1], Bonnie M Weasner[1], Kevin R Cook[1], Michael J Stinchfield[2], Shu Kondo[3], Kuniaki Saito[4], Justin Kumar[5], Stuart J Newfeld[2] [1]Biology, Indiana University, [2]Arizona State University, [3]Tokyo University of Science, [4]National Institute of Genetics, [5]Indiana University

As part of an ongoing effort to generate comprehensive resources for the experimental analysis of fourth chromosome genes in *Drosophila melanogaster*, the Fourth Chromosome Resource Project has used CRISPR mutagenesis with single guide RNAs to isolate mutations in 62 of the 80 fourth chromosome, protein-coding genes. These mutations were induced on a fourth chromosome bearing the basal FRT101F insertion to facilitate experimental approaches involving FLP recombinase-induced mitotic recombination. To permit straightforward comparisons among mutant stocks, most of the mutations were generated on isogenic fourth chromosomes, which were then crossed into a common genetic background. Of the 119 mutations, 84 are frameshift mutations likely to be null alleles, 29 are small in-frame deletions and 6 have yet to be characterized molecularly. The mutations were tested for recessive lethal, female sterile and visible phenotypes. Interestingly, our data is comparable to reports from Hochman in the 1970's regarding the number of lethals on the 4th. Stable stocks for most of the mutations have been submitted to repositories in the United States and Japan for public distribution.

898T **New TRiP resources for gene expression and protein detection** Jonathan Zirin[1], Ben Ewen-Campen[1], Justin A Bosch[2], Ah-Ram Kim[1], Raphael Lopes[1], Barbara Jusiak[3], Lu-Ping Liu[1], Christians Villalta[1], Alexandria Risbeck[1], Elizabeth Filine[1], Corey Forman[1], Yanhui Hu[1], Stephanie Mohr[1], Norbert Perrimon[1,4] [1]Harvard Medical School, [2]University of Utah School of Medicine, [3]University of California, Irvine, [4]HHMI

The Transgenic RNAi Project (TRiP) is an in vivo functional genetics platform that has developed over 20,000 shRNA and sgRNA fly stocks for the research community. Distributed by the Bloomington Drosophila Stock Center (BDSC), these resources offer powerful and versatile tools for gene knockdown, knockout, and activation. Researchers can now readily access fly stocks to modulate gene expression ("dial down" or "dial up") across various developmental stages and tissues. Here we describe new in vivo resources designed to enable: 1) Dual binary system usage in Drosophila tissues. We created over 40 LexA-GAD and QF2 insertions using CRISPR knock-in technology, focusing on genes with well-characterized GAL4 expression patterns. These insertions were validated for tissue specificity in larvae; 2) To validate scRNA gene clusters, we are generating Gal80-repressible split-GAL4 knock-in lines targeting glial cell clusters. Using an algorithm to identify the smallest unique set of marker genes defining each cluster, these lines enable precise validation of specific glial subtypes; 3) detection of fly proteins. We are tagging the C-terminus of over 300 high-confidence Drosophila orthologs of human mitochondrial disease genes with a NanoTag epitope. This allows for protein detection using a high affinity nanobody against the NanoTag. Together, these fly lines will provide a set of tools for orthogonal activation or repression of different genes, highly specific drivers for glial cell subtypes, and expanded capabilities for detecting fly mitochondrial proteins.

899T **Drosophila Laboratory Pangenome Database: A collection of reference genome assemblies of popular *D. melanogaster* laboratory strains** Trevor Millar[1], Mahul Chakraborty[2] [1]Interdisciplinary Graduate Program in Genetics and Genomics, Texas A&M University, [2]Department of Biology, Texas A&M University

The whole genome shotgun sequencing of the *Drosophila melanogaster* reference genome ISO1 represents a landmark in genetics and genomics. The ISO1 sequence has served as the reference sequence for community projects such as modENCODE and laboratory experiments seeking to delineate the functions of a single gene or large genomic regions. However, most *D. melanogaster* strains used in laboratories worldwide, including wild-type and transgenic strains, have diverse genetic backgrounds. Genetic variation, particularly structural variation (SV), between the reference strain and the other strains may confound interpreting genotype-phenotype relationships using non-reference strains. To evaluate this reference bias, we generated extremely contiguous reference genome assemblies of 11 popular *D. melanogaster* strains, including the genetic background of strains used in deletion mapping, reporter assays, RNAi, genome editing, as well as wild-type strains (e.g., Oregon-R, w1118, Canton-S). We also used Hi-C contact frequency from two strains to assemble an X, a second, and a third chromosome balancer chromosomes, FM7a, CyO, and TM6B, respectively. We developed an improved version of Structural Variants from Mummer (**SVMU**), a software for SV annotation between genome assemblies, to reveal a comprehensive map of SVs between the new assemblies and the reference strain ISO1. We find extensive SVs in protein coding and noncoding regulatory elements, potentially confounding effects of gene targeting or genome modifications. We further show that genetic variation in a popular strain used in CRISPR-Cas9 experiments affects many CRISPR target sites predicted based on the reference genome ISO1, potentially affecting genome editing efficiency. Our results highlight the urgent need for a database of reference genomes of laboratory strains. Thus, we introduce the Drosophila Laboratory Pangenome Database (DLPD), a collection of ever-growing reference genome assemblies of popular *D. melanogaster* laboratory strains available at https://github.com/chakrabortymlab/DLPD.

900T **A self-eliminating allelic-drive reverses insecticide resistance in *Drosophila* leaving no transgene in the population** ANKUSH AURADKAR[1], Rodrigo M. Corder[2], John M. Marshall[3], Ethan Bier[4] [1]Biological Sciences, University of California, San Diego, [2]University of São Paulo, [3]University of California, Berkeley, [4]University of California, San Diego

Insecticide resistance (IR) poses a significant global challenge to public health and welfare. Here, we develop a locally-acting unitary self-eliminating allelic-drive system, inserted into the *Drosophila melanogaster yellow* (*y*) locus. The drive cassette encodes both Cas9 and a single gRNA to bias inheritance of the favored wild-type (1014L) allele over the IR (1014F) variant of the *voltage-gated sodium ion channel* (*vgsc*) target locus. When enduring a fitness cost, this transiently-acting drive can increase the frequency of the wild-type allele to 100%, depending on its seeding ratio, before being eliminated from the population. However, in a fitness-neutral "hover" mode, the drive maintains a constant frequency in the population, completely converting IR alleles to wild-type, even at low initial seeding ratios.

901F **Functional characterization of species-specific neonicotinoid response using chimeric nicotinic acetylcholine receptor (nAChR) subunits in a *Drosophila* model** Anna K Lassota[1], Irmgard U Haussmann[1,2], Thomas C Dix[1], David W.J. McQuarrie[1], Veronica Dezi[1], Abdullah I Hans[1], Roland Arnold[3,4], Matthias Soller[1,5] [1]School of Biosciences, College of Life and Environmental Sciences, University of Birmingham, [2]College of Life Science, Birmingham City University, [3]Institute of Cancer and Genomics Sciences, College of Medical and Dental Sciences, University of Birmingham, [4]Birmingham Centre for Genome Biology, University of Birmingham, [5]Division of Molecular and Cellular Function, School of Biological Sciences, University of Manchester

Nicotinic acetylcholine receptors (nAChRs) are essential for neuronal function and synaptic plasticity. Neonicotinoids, a class of neurotoxic pesticides, target nAChRs in insects and pose significant threats to pollinators such as honeybee. Neonicotinoids have a species-specific sensitivity, with bees exhibiting high susceptibility and *Drosophila melanogaster* showing greater resistance.

To investigate whether this difference in susceptibility stems from variations in nAChR subunit composition, we analysed the sequence conservation of nAChR subunits across species. Remarkably, we found a high degree of conservation between the honey bee nAChR α8 and the *Drosophila* nAChR β2 subunits, differing only in a few amino acids within the ligand-binding domain. To test whether these differences underlie the relative resistance of *Drosophila* to neonicotinoids, we replaced the ligand-binding domain of β2 in *Drosophila* with α8 of honey bees by CRISPR-Cas9 gene editing using our optimized PlatinumCRISPr system (Haussmann *et al*., 2024). These flies exhibited impaired motor functions, including reduced climbing ability and disrupted flight, compared to wild-type controls. Moreover, the response to neonicotinoids is altered. Our chimeric nAChRs subunits provide a means to test cross-species toxicity and reveal how subtle differences in receptor structure can significantly impact on motor function and pesticide response.

Haussmann, I.U., Dix, T.C., Mcquarrie, D.W.J., Dezi, V., Hans, A.I., Arnold, R., Soller, M.: Structure-optimized sgRNA selection with PlatinumCRISPr for efficient Cas9 generation of knock-outs. Genome Research, in press.

902F **An efficient and universal single-cell transcriptomic analysis framework for cell-type-specific labeling and manipulation** Yen-Chung Chen, Yu-Chieh David Chen, Claude Desplan Biology, New York University

Recent advances in high-throughput profiling have revolutionized our understanding of cellular diversity with unprecedented resolution. Numerous novel cell types have been discovered in health and diseases. Genetic access to these ever-expanding molecular cell types is essential not only for visualizing these cell types *in vivo* but also for deciphering the functions of genes expressed in these cell types. While the wealth of genetic tools available in Drosophila has enabled sophisticated cell-type-specific genetic manipulation, it is still challenging to target novel cell types that are only defined by transcriptomes: First, genetic drivers based on enhancers do not always reproduce the expression of a specific gene and are therefore hard to select based on gene expression. Secondly, genetic drivers like the Gal4/UAS system are binary and might not reflect quantitative differences of gene expression. Finally, one marker is rarely sufficient to identify a unique cell type and instead labels multiple cell types. There is therefore a need for a method that finds single or combinations of binary markers that are predictable from single-cell transcriptomic datasets and optimized for their joint specificity when used in intersection.

We previously reported a method to predict marker combinations for neuronal types in the developing fly visual system. We generated genetic drivers and showed that specific neurons are targeted as predicted across developmental stages. While the method performed similarly well in many datasets, we noticed that it had difficulties with datasets with mixed clusters containing multiple cell types. The limitation was caused by the algorithm treating each cluster as a homogenous sample. To make the algorithm applicable to all single-cell transcriptomic datasets, we refined the method to model expression state at the single-cell level instead of at the cluster level. This allowed the algorithm to perform consistently regardless of the number of clusters and be robust to clusters with mixed cell types. Users can select for optimal clustering resolution based on experimental results with marker combinations defined at different resolutions. Our marker finding method is available as an R package with step-by-step documentation. We also devised a web application to interactively visualize and explore marker combinations and host a repository of existing gene-specific split-Gal4 reagents to streamline resource sharing and collaboration. The cell-type-specific markers identified using our approach will enable the design of split-GAL4 and Flp-out lines for the genetic manipulations of specific cell-types.

903F **Harnessing Truncated gRNA (tgRNA) Targeting the Cas9 Promoter to Enhance CRISPR-Based Homing Gene Drive Performance** Lei Yang, Ethan Bier Department of Cell and Developmental Biology, University of California, San Diego

CRISPR-based homing gene-drives are increasingly being explored as a practical tool for combating vector-borne diseases through population modification or suppression. Their ability to rapidly propagate desired genetic changes via super-Mendelian inheritance is a significant advantage. However, a major challenge lies in the maternal deposition of Cas9 expression, which can lead to the formation of resistance alleles and severely hinder the efficacy of CRISPR-based homing gene drives. While the *vasa* promoter has been commonly used to restrict Cas9 expression to the germline in gene-drives for *Drosophila* and other insect species, maternal Cas9 deposition still results in considerable resistance allele formation. In this study, we present a novel strategy to limit maternal deposition of Cas9 expression by utilizing a truncated gRNA (tgRNA) that targets the *vasa* promoter. This 14 nucleotide (nt) tgRNA-y can complex with Cas9 to bind its target site with great specificity, but does not lead to DNA cleavage. To enable sequence specific binding of the tgRNA-y to the *vasa* promoter, we introduced several nucleotide changes in the region between the *vasa* TATA box and the transcription start site (TSS). Our results show that both the mutated vasaCas9 (MvasaCas9) alone and the combination of MvasaCas9 with tgRNA-y exhibit homing gene drive efficiencies (91.5-94.3%) comparable to those of the unmodified vasaCas9 (88.7-92.2%) in both maternal and paternal crosses with full-length (20 nt) gRNA-y1. We also quantified Cas9-mediated target cleavage in somatic cells of F1 trans-heterozygotes using next-generation sequencing, and found that significantly fewer NHEJ events were generated in the presence of both MvasaCas9 and tgRNA-y compared to controls. Additionally, we tested the effect of combining tgRNA-y and MvasaCas9 with a split-drive element inserted at the yellow locus that carryies the full-length gRNA-y1 under conditions where transmission is greatly attenuated due to strong maternal transmission of Cas9/gRNA complexes. We found that the MvasaCas9 and tgRNA-y combination outperformed the unmodified vasaCas9 control in these maternal crosses, increasing the homing gene drive efficiency from 80.6% to 92.4%, while maintaining high drive efficiency in paternal crosses (93.3%). Overall, these findings demonstrate that tgRNA can effectively mitigate the maternal deposition of Cas9 and significantly enhance the performance of CRISPR-based homing gene drives.

904F **Deterministic Genetic Barcoding for Multiplexed Behavioral and Single-Cell Transcriptomic Studies** Jorge B Mendana[1], Margaret Donovan[1], Lindsey G O'Brien[1], Benjamin Auch[1], John Garbe[1], Daryl M Gohl[1,2] [1]University of Minnesota Genomics Center, University of Minnesota, [2]Department of Genetics, Cell Biology, and Development, University of Minnesota

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 describe 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 polyadenylation 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 including positive identification of cell types in cell atlas projects, identification of multiplet droplets, and barcoding of experimental timepoints, conditions, and replicates. 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 has the potential to enable large-scale behavioral screens in addition to improving the ability to reliably annotate cell atlas data, expanding the scope, and improving the robustness of single-cell transcriptomic experiments.

905F **Drosophila three-dimensional cell cultures** Arthur Luhur[1], Daniel Mariyappa[2], Andrew Zelhof[3] [1]Indiana University Bloomington, [2]Biology, Indiana University, [3]Biology, Indiana University Bloomington

Ever since the first Drosophila cell line was established over fifty years ago, Drosophila cells in culture have been a valuable tool in studies involving signal transduction, receptor ligand interaction, circadian biology, cellular stress response, neuronal processes, cellular homeostasis and metabolism, innate immunity, and in functional genomics high-throughput screens. These studies have benefitted by the increase in the availability of cell lines with over 200 unique cell lines currently cataloged at the current Drosophila Genomics Resource Center (DGRC). However, the amenability of Drosophila cells to three-dimensional (3D) cell culture has not been systematically characterized. Being able to grow cells in 3D provides additional adds to the utility of cell lines by providing a means to investigate cellular mechanisms governing cell polarity, differentiation, cell shape changes, and changes in gene expression.

We tested a panel of 10 Drosophila cell lines (embryonic-, ovarian-, wing disc-or larval central nervous system-derived) for their suitability to form 3D cultures using either extracellular matrix (ECM)- or hanging drop-based methods. With either of the methods, several embryonic and ovarian cell lines form spheroid structures that have a smooth outer surface. Cell lines capable of forming the 3D spheroids also exhibit differential membrane localization of adherens junction components, DE-Cadherin and Armadillo in the medium exposed cells (outer cells-OC) as opposed to cells deeper (inner cells-IC) in the spheroids in the spheroid. Going forward, we will determine whether response to transfection or drugs differ between IC's and OC's. Establishing reproducible protocols for Drosophila 3D cell culture and their characterization will provide additional tools for the Drosophila community to investigate complex cellular interactions in vitro.

906F **Predictive generation of type-specific enhancer-Gal4 drivers for developing neurons** Rose Coyne, McKenzie Treese, Cathleen Lake, M. Neşet Özel Stowers Institute for Medical Research

Targeted genetic access to specific neuronal types is crucial for understanding neural circuit development and function; but the extreme cell-type diversity of brains necessitates extremely specific genetic tools. The Janelia and VT-Gal4 collections have provided genetic access to hundreds of new cell types in recent decades; intersectional methods (split-Gal4) in particular can deliver very specific reporters. But the vast majority of these tools are not expressed (or lose their specificity) in developing neurons; and most cell-types in the fly brain remain genetically inaccessible. While MiMIC/CRIMIC approaches enable targeted generation of gene traps guided by transcriptomic atlases, including during development, the genes that are expressed in only one (or even a few) cell-type are very rare. Alternatively, recent snATAC-seq studies have shown that differential chromatin accessibility can be used to predict enhancers specifically active in restricted cell types. As the same genes are often regulated by different cis-regulatory regions in different cell-types, we hypothesized that highly specific enhancer reporters can be predictably produced for most neurons. We generated a large single-nucleus RNA and ATAC-seq (multiomics) atlas of developing Drosophila optic lobes at 4 stages (P0, P24, P48, Adult). The integrative analysis of two modalities exceeded the cell-type resolution of previously published scRNA-seq datasets, with 259 distinct clusters uniquely resolved throughout development and >100 confidently annotated to specific cell types in the visual system. We developed a computational framework that leverages the multiome data to identify highly specifically accessible regions for each cell-type across stages, and to prioritize active elements based on correlations of their accessibility with expression of nearby genes. We produced a pilot batch of 10 enhancer-Gal4s for distinct neuronal types in the visual system, 6 of which were expressed in the predicted cell type in at least 2 developmental stages. This also revealed that enhancer activity is strongly dependent (in 4/10 candidates) on its directionality (cloning orientation), an unexpected feature for cis-regulatory elements. Molecular mechanisms underlying this phenomenon remain to be determined. In summary, our results establish a proof-of-concept for targeted generation of developmental Gal4 drivers for virtually any cell type in the visual system, which can also be applied to other tissues.

907F **Applying Deep Learning Models to Derive Pose Estimation for Behavioral Analysis** Elizabeth Miller, James Lee, Sarah Clark Neuroscience Institute, Georgia State University

The amount of time it takes a researcher to annotate and quantify animal behavior has long been a major barrier to high-throughput behavioral analysis. There have been many recent advances in open-source machine learning models through platforms like TensorFlow, which has significantly accelerated the development of pose estimation models based on deep learning. These advancements lower the barrier to entry for scientists to not only help in understanding animal behavior through object movement tracking, but also open new avenues for behavioral research. To study the behavior of Drosophila using videos of the forced swim test, we employed the pose estimation software SLEAP. This software allows users to analyze multiple animals simultaneously, greatly reducing analysis time. Here, we describe the pipeline we developed to address various research questions related to behavior analysis and present a comparison of the accuracy of analysis via the pipeline compared to manual behavior annotation.

908F **Oracle, a High Throughput Data Aggregation and *in silico* Analysis Pipeline** Jacie Cheng, Ryan O›Neill, Nasser Rusan Cell and Developmental Biology Center, Cell and Developmental Biology Center, National Heart Lung and Blood Institute, National Institutes of Health

Scientists leverage databases like FlyBase, MARRVEL, and NCBI to generate hypotheses about genes and proteins of interest, but a major bottleneck remains-- manually compiling data is labor and time intensive and susceptible to human error. To address these challenges, the Oracle pipeline aims to streamline hypothesis generation by aggregating information across major biological databases, automating *in silico* protein-protein interaction assays, and gathering additional information to suggest downstream assays. Ongoing efforts focus on using Oracle to improve rare disease modeling involving unknown and novel genes. To illustrate its utility, we present a case study of pericentrin (PCNT) and its *Drosophila* ortholog Pericentrin-like Protein (PLP). Galleta et al. (2014) hypothesized that mutations in PCNT lead to microcephalic osteodysplastic primordial dwarfism type and sought to understand it better by using the highly conserved PACT domain found in *Drosophila* PLP. After ingesting the associated gene identifier, a desired isoform, and possible mutations, Oracle aims to suggest the best *Drosophila* ortholog, run a ColabFold-based protein-protein interaction screen, and collect information from literature and high-throughput expression studies to rank possible experiments to perform. The initial results of its *in silico* protein interaction screen align with the results of Galleta et al. Oracle is in the process of revolutionizing scientists' lab experience by expediting their journey to the bench, enhancing accessibility to *in silico* assays, and facilitating high throughput studies on genes with unknown functions to drive discovery forward

909F **Proteomic mapping of organ secretomes using in vivo proximity labeling** Justin A Bosch[1,2], Pierre Michel Jean Beltran[3], Cooper Cavers[2], Thai LaGraff[2], Randy Melanson[3], Ankita Singh[2], Weihang Chen[2], Yanhui Hu[2], Sudhir Tattikota[2], Ying Liu[2], Yousuf Hashmi[2], Steven Carr[3], Norbert Perrimon[2,4] [1]Human Genetics, University of Utah, [2]Harvard Medical School, [3]Broad Institute, [4]Howard Hughes Medical Institute

Identifying an animal's complete set of secreted proteins (the "secretome"), as well as deciphering their tissues of origin, is extremely challenging. To address this, we developed a proteomic approach, involving proximity labeling (TurboID) and mass spectrometry, to identify blood plasma proteins derived from specific cell-types and organs in *Drosophila melanogaster* larvae. We identified 540 proteins from 10 major cell/tissue types (e.g. muscle, adipose, glia), including most known fly blood proteins. We confirmed the quality of this dataset, using a combination of single cell RNA (scRNA) sequencing and CRISPR/Cas9 knock-in reporter lines. We identified hundreds of uncharacterized secreted proteins, many of which originate from a single cell-type/tissue, including some from less appreciated sources (e.g. glia, oenocytes). In addition, we discover proteins that are deposited in a different tissue than where they are synthesized, suggesting travel through circulation and potential inter-organ functions. For example, we show that CG6867, the single fly ortholog of mammalian Olfactomedins, is secreted from muscle and integrates into the basement membrane of distal tissues. We believe that our secretome map will serve as a resource to investigate blood protein function, discover novel tissue-tissue communication signals, and compare with homologues of human biomarkers.

910F **A *Drosophila* holidic diet optimised for growth and development** Sebastian Sorge[1], Victor Girard[1], Lena Lampe[1], Vanessa Tixier[2], Alexandra Weaver[1], Theresa Higgins[1], Alex Gould[1] [1]The Francis Crick Institute, [2]Université Clermont Auvergne

Diets composed of chemically pure components (holidic diets) are useful for determining the metabolic roles of individual nutrients. For the model organism *Drosophila melanogaster,* existing holidic diets are unable to support rapid growth characteristic of the larval stage. Here, we use a nutrient co-optimisation strategy across more than 50 diet variants to design HolFast, a holidic medium tailored to fast larval growth and development. We identify dietary amino acid ratios optimal for developmental speed but show that they compromise survival unless vitamins and sterols are co-optimised. Rapid development on HolFast is not improved by adding fatty acids but it is dependent upon their *de novo* synthesis in the fat body via *fatty acid synthase* (*FASN*). HolFast outperforms other holidic diets, supporting rates of growth and development close to those of yeast-based diets and, under germ-free conditions, identical. HolFast has wide applications in nutritional and metabolic studies of *Drosophila* development.

911F **Enhancer scanning mutagenesis of the *apterous* regulatory region** Andrew Alegria, Jorge Blanco Mendana, Margaret Donovan, Daryl Gohl University of Minnesota

Deep mutational scanning experiments have been used to characterize the functional landscape of proteins and to identify transcriptional regulatory elements. However, such experiments are typically carried out in isolated cell suspensions such as yeast or cell culture, due to the difficulty of generating sufficient numbers of transformants. This constraint means that mutational scanning approaches have only rarely been applied to query developmental processes in multicellular animals. We used an automated microinjection robot to carry out a large-scale CRISPR enhancer scanning mutagenesis screen of the 27 kb *apterous* regulatory region, a gene involved in wing development in Drosophila. Previous work has identified regulatory elements and transcription factor binding sites that control *apterous* expression throughout development to pattern the dorsal compartment in the developing wing imaginal disc, providing a detailed ground truth for assessing the results of the enhancer scanning mutagenesis screen. We injected a pool of all possible guide RNAs (containing an *S. pyogenes* PAM sequence) and isolated thousands of transformants which were crossed to a germline-expressing Cas9 strain and screened over an *apterous* enhancer deletion. We identified dozens of null and hypomorph alleles and sequenced the corresponding guide RNA and *apterous* lesion. The enhancer scanning mutagenesis screen identified the known *apterous* enhancer elements and even pinpointed transcription factor binding sites, demonstrating that high-throughput transgenesis paired with next-generation sequencing is an efficient approach for detailed characterization of complex regulatory regions.

912S **A novel CRISPR/Cas9 toolkit for tissue-specific mutagenesis in Drosophila** Xinchen Chen[1], Sarah Perry[2], Ziwei Fan[1], Bei Wang[1], Elizabeth Loxterkamp[3], Shuran Wang[1], Jiayi Hu[1], Dion Dickman[3], Chun Han[1] [1]Cornell University, [2]Austin Peay State University, [3]University of Southern California

The Drosophila larval neuromuscular junction (NMJ) is a powerful system for studying synaptic development, function, and plasticity, but genetic investigations have been limited by the inability to target essential genes or address pleiotropic effects during knockdown in multiple tissues. While RNAi allows for gene knockdown in specific cell types, it rarely achieves complete gene loss of function. Here, we present a CRISPR-mediated tissue-restricted mutagenesis (CRISPR-TRiM) toolkit for efficient somatic gene knockout in the three principal cell types of the Drosophila NMJ: motoneurons, muscles, and glial cells. We developed this toolkit by converting various NMJ GAL4 driver lines into corresponding Cas9 lines, enabling tissue-specific expression of Cas9 in neurons, motor neurons, muscles, and glia. Additionally, we optimized multiplexed guide RNA (gRNA) vectors for targeted mutagenesis of key synaptic genes. This approach allows for efficient gene knockout, with some genes showing over 95% reduction in protein levels at specific tissues at the NMJ and exhibiting expected mutant phenotypes electrophysiologically. This method can be used for the simultaneous knockout of multiple genes, potentially up to 8 genes in a single tissue. This CRISPR-TRiM toolkit is a valuable resource for investigating gene function at the Drosophila NMJ, enabling tissue-specific combinatorial genetic analysis that was previously difficult to achieve.

913S **A template for aligning images of the larval ventral nerve cord** Peter Newstein[1], Chris Q Doe[2] [1]University of Oregon, [2]HHMI

The availability of transmission electron microscopy (TEM) volumes, and databases of various light microscopy images has accelerated the pace of neuroscience research in the adult Drosophila brain. These advances depend on template brains, which are used to align images for multiple sources onto the same coordinate system. Although there are excellent templates for the adult central nervous system, similar templates for the larval central nervous system are lacking. In particular, there is no template for the ventral nerve cord (VNC) of newborn larvae. This gap in resources is significant due to the volume of research, particularly in neurodevelopment, that is focused on the embryonic-born VNC neurons.

The ability to map brain images onto each other has a variety of uses in neuroscience. One common use is to directly compare the brains of different animals. For example, in experiments where random subsets of neurons are labeled, template brains can be used to combine multiple replicates to study the full set of neurons. Alternatively, in experiments where a manipulation may affect the population of labeled neurons, control and experimental images can be aligned onto the same template in order to directly compare expression. Finally, templates can be used to map light images onto TEM volume. Over the last several years, A TEM volume of a 6 hour old larva has been annotated with neuron types. The utility of this dataset has been limited by a lack of automated comparison between light images taken in various labs and the TEM volume. This template makes this comparison easier.

A particular challenge of the larval VNC, is that the neuropils are relatively indistinct compared to intricate structures found in the adult neuropils. This limits the precision of the alignment that can be achieved using only the neuropil as a landmark. Instead, this work leverages the tracts of axons that extend down the VNC to precisely align images. These tracts of axons can also be located within the TEM volume, making it possible to align a light microscopy image to the TEM volume with this added precision.

This poster describes the process of creating a template brain from light microscopy images, creating an analogous image in the TEM volume, and aligning these images together. It also demonstrates its use in newly acquired light images. We have made all relevant code publicly available, and we believe this represents a valuable resource for the field.

914S **Genome-wide CRISPR Screening Reveals Novel Regulators of Key Signaling Pathways** Zhongjie Zhang[1], Raghuvir Viswanatha[1], Yanhui Hu[2], Stephanie E Mohr[1], Norbert Perrimon[1,3] [1]Department of Genetics, Harvard Medical School, [2]Harvard Medical School, [3]Howard Hughes Medical Institute

Key signaling pathways, including Wnt-Wingless, JAK/STAT, JNK, Hedgehog, and Hippo, are essential for regulating cellular processes and maintaining homeostasis. Despite significant advances in understanding these pathways in recent years, the full spectrum of their regulatory mechanisms remains incomplete. In this study, we employed a genome-wide CRISPR-based knockout approach to uncover novel regulators of these pathways. Our screen identified 233 and 166 potential regulators of the Wnt-Wingless and JAK/STAT pathways, respectively. Comparison with our previous RNAi-based screen revealed limited overlap in identified regulators, although top-ranked genes showed strong consistency between the two methods. Notably, the CRISPR screening approach identified a greater number of known pathway components, indicating enhanced screening efficiency. We are currently validating these potential regulators in vivo in *Drosophila*. In summary, we established a robust genome-wide CRISPR screening platform in *Drosophila* cells to advance the study of regulatory components in signaling pathways, which may pave the way toward the identification of new therapeutic targets and enhance our understanding of cellular regulation in both health and disease.

915S **CRISPR-mediated allelic correction of Cystic Fibrosis mutations in *Drosophila* using the homologous chromosome as repair template (HTR)** Matthew Le Roy[1], Annabel Guichard[1], Helena Araujo[2], Ethan Bier[1] [1]University of California San Diego, [2]Universidade Federal do Rio de Janeiro

Cystic Fibrosis is a recessive genetic disorder caused by mutations in the Cystic Fibrosis Transmembrane conductance Regulator (CFTR) gene, which encodes a Chloride ion channel. Disease symptoms include lung and digestive dysfunctions, caused by the buildup of mucus resulting from impaired fluid-coupled chloride secretion.

In a previous study from the Perrimon group, CG5789 was identified as the *Drosophila* ortholog of CFTR. In that study, the authors showed that changes in several physiological parameters characteristic of cystic fibrosis can be induced by CG5789 RNAi. In addition, human CFTR -but not its mutant counterparts- expressed in the midgut could rescue these phenotypes. These findings indicate that *Drosophila* provides a suitable model system for Cystic Fibrosis studies.

Our goal is to perform *in vivo* allelic correction of mutant CFTR alleles via Homologous chromosome Templated Repair (HTR), wherein the homologous chromosome provides the template for correcting the disease-causing allele in *Drosophila*. For this purpose, we are using established mutant UAS-hCFTR transgenic lines, inserted at the same genomic location. Previous work from our group showed that similar allelic correction at *white* can reach up to 30% when using Cas9, and 50% when using the Cas9D10A Nickase. We have designed gRNA targeting F508del, the most common Cystic Fibrosis mutation in humans, as well as gRNAs targeting other known mutations, such as Y122X, W1282X and N1303K.

We hope to capture and quantify this repair through high throughput next generation DNA sequencing and immunofluorescence staining of the digestive system. This work will allow us to answer the following questions: How does Nickase-induced repair differ from Cas9-induced repair at the CFTR locus? How does the repair efficiency vary depending on the targeted mutation and corresponding gRNA? Is intestinal stem-cell-specific correction more efficient than correction in enterocytes? Can two gRNAs targeting two trans-heterozygous mutations act synergistically or additively to correct hCFTR function? Results will be presented.

916S **Genome wide elucidation of cis-regulatory elements and gene regulation in fly aging and pro-longevity** Bo Sun[1], Bo Sun[2], Tyler Jackson[2], Tzu-Chiao Lu[2], Yejin Park[2] [1]Huffington Center On Aging, Baylor College of Medicine, [2]Baylor College of Medicine

Aging is a complex biological process marked by progressive functional decline, with gene regulation playing a crucial role in mediating age-related changes across tissues. In this study, we perform a genome-wide elucidation of cis-regulatory elements (CREs) and gene regulation mechanisms associated with aging and pro-longevity in Drosophila melanogaster. With single-nuclear and multi-omic approaches, we mapped cis-regulatory regions across the fly genome, identifying enhancers, promoters, and other regulatory elements that modulate gene expression in aging and longevity-promoting contexts by low temperature. We integrated chromatin accessibility and transcriptome data from young to aged flies to build a comprehensive landscape of CRE activity and transcription factor binding dynamics across different tissues and cell types. By characterizing regulatory networks and transcription factor motifs that control age-related and pro-longevity gene expression, we uncovered specific regulatory mechanisms that drive tissue resilience and lifespan extension. With combined aging features revealed on chromatin and transcripts level, we provide an unprecedented detailed and informative biological aging and pro-longevity map.

917S **Auxin-Inducible Degron Mediated Tissue Specific Degradation of Endogenous Proteins in Drosophila** Trisha Mynampati[1], Wanpeng Wang[2], Tom Kornberg[2] [1]University of California, Berkeley, [2]University of California, San Francisco

The plant hormone auxin directly induces binding between the auxin receptor F-box proteins TIR1/AFB and AUX/IAA family of transcription factors and leads to the degradation of the AUX/IAA proteins. Other eukaryotes lack the auxin receptors but share the SCF E3 ubiquitin ligase degradation pathway. Previous research in human and chicken cell lines, mouse models, and fly auxin-dependent gene expression studies have demonstrated that transplanting the TIR1/AFB - AUX/IAA system into non-plant cells allows for controlled protein degradation following auxin addition. Rapid depletion of target proteins and temporal control avoids accumulating effects and permits observation of immediate outcomes. Such precision is suitable for studying signaling pathways where the processes are intrinsically transient and are sensitive to protein level changes. Controlled degradation is also valuable for studying the functions of proteins that produce a lethal phenotype when knocked out in vivo.

We adapted the auxin-degron system to *Drosophila melanogaster* and systematically evaluated different degron tags, the addition of auxin response transcription factor (ARF), and the effectiveness of TIR1 mutants combined with an auxin derivative. We recommend using the full-length IAA protein with ARF expression to suppress background degradation and using the miniAID tag for easier CRISPR knock-in and minimal impact on protein function. The F74A mutation in the TIR1 auxin binding pocket coupled with the auxin derivative 5-Ad-IAA rapidly depletes the protein of interest (POI) with low chemical concentrations. Intronic *Minos* mediated integration cassettes (MiMIC) with modified double headers and CRISPR knock-ins are utilized for effective endogenous gene tagging. We demonstrate these methods using two F-box proteins, OsTIR1 and AtAFB2, and their mutants, OsTIR1^{F74A} and AtAFB2^{F74A}, paired with auxin IAA or auxin derivative 5-Ad-IAA respectively. Although AtAFB2 maintains low basal degradation in other model systems, empirical data demonstrates that OsTIR1 and AtAFB2 have similar background degradation rates in *Drosophila*. We showcase successful spatial and temporal depletion of the POI in the wing disc and brain and discuss considerations to leverage this approach in *Drosophila* studies.

918S **Standardizing and Streamlining Drosophila melanogaster meta-analysis with MetaAtlas** Andrew D Gillen[1], Shannon Keenan[2], Julian Dow[3] [1]MVLS Shared Research Facilities, University of Glasgow, [2]University of Strathclyde, [3]University of Glasgow

In the age of “Big Data”, our capacity to generate large datasets is increasing at an astronomical rate. This is especially true within the model organisms where much research is focused.

A flashpoint for this issue exists in Drosophila melanogaster research, with over 1,900 independent groups around the world studying the fly. In such a wide field, it is unsurprising that vast quantities of data exist – indeed, NCBI’s Sequence Run Archive (SRA) currently hosts >90,000 individual Drosophila RNAseq samples, divided between single cell and bulk RNAseq data.

Intuitively, this is a good thing – after all, more data should enable researchers to perform deep preliminary studies on public data to generate new hypothesis. However, the sheer volume of available data, and the relative paucity of associated metadata, makes navigating the data landscape an onerous task. In addition, data from diverse groups or produced in different ways show wildly varying results, even for the most high-profile datasets.

To rectify this issue, we have created a new, open resource for the fly community, MetaAtlas (www.metaatlas.org), which plays host to harmonized analyses of all interpretable SRA bulk RNAseq datasets (>20,000 individual samples). Manually curated metadata allows samples to be easily sorted by various categories, including fly genotype, life stage or experimental intervention. Selected experiments can then be directly compared, providing a rapid readout of gene expression values from across the published research space.

By streamlining meta-analysis, MetaAtlas provides users with access to the full power of the Drosophila melanogaster community, enabling Big Data to become a key strength of, rather than an obstacle to, analysis.

919S **DRSC Bioinformatics: New and improved online resources for *Drosophila* research** Claire Hu, Mujeeb Qadiri, Ah-Ram Kim, Aram Comjean, Chenxi Gao, Jonathan Zirin, Norbert Perrimon, Stephanie E. Mohr Harvard Medical School

The bioinformatics team at the Drosophila RNAi Screening Center (DRSC) builds informatics tools with the goal of supporting research in Drosophila and other model organisms, as well as an increasing number of non-model species. We work to maintain our core suite of tools, including our popular DIOPT approach to ortholog identification, and regularly expand in new directions.

The use of artificial intelligence (AI)-based approaches such as AlphaFold has revolutionized protein structure prediction. AlphaFold-Multimer (AFM) builds on this by predicting protein-protein interactions (PPIs). Moreover, Drosophila is an excellent model in which to test the quality and biological implications of predicted PPIs. To support this, we built a pipeline that assesses protein-protein interactions (PPIs) predicted using AFM and created an online resource, FlyPredictome, to store the results and related information. At FlyPredictome, users can search and view AFM-generated predictions and evaluations, as well as retrieve predicted structures and other relevant information, for over 100,000 Drosophila protein pairs.

FlyPhone allows users to mine scRNA-seq data for potential cell-cell communication events occurring between cells in different clusters. We have now supplemented the manually curated ligand-receptor relationships from FlyPhone vs1 to include more than 600 curated ligand-receptor pairs mapped from mammalian data and supported by AFM prediction. Furthermore, we optimized the algorithm underlying FlyPhone for better performance, accuracy, and ease of use. Moreover, the updated resource, FlyPhone vs2, now supports comparison of cell-cell communication between different samples, making it possible identify potential condition-specific signaling events.

We have also made other updates. For example, we have expanded to provide an increasing number of online resources relevant to arthropod vectors of human diseases. In addition, we developed the Cross-Species Epitope Sequence Analysis (CESA) approach for discovery of existing phospho-specific antibodies that were made to target for mammals but have potential to recognize conserved phospho-sites in proteins from model species.

Altogether, our bioinformatics suite provides Drosophila researchers and others with online tools relevant to all steps in the scientific discovery process and facilitate a broad range of use-cases.

920S **Are Two Cells Better Than One? TubAtlas: A Single-Nuclei Gene Expression Map of the Insect Renal System** Karen McLuskey[1], Sue Ann Krause[2], Andrew Gillen[2], Anthony Doran[2], Keqin Li[2], Virginia Howick[2], Amelia Cox[2], Pawel Herzyk[2], Julian A Dow[2] [1]School of Molecular Biosciences, University of Glasgow, [2]University of Glasgow

The insect renal system is integral to the remarkable ecological success of the species. By helping to maintain homeostasis through water conservation, ion balance, and efficient waste removal, it enables insects to exploit habitats ranging from bone-dry deserts to tidal estuaries, spanning ecosystems from the equator to the Antarctic.

To transform our understanding of renal function across diverse insect orders, we combined bulk and single-nuclei transcriptomics (snRNA-seq) with the aim of producing a unified, public-domain atlas (TubAtlas). In snRNA-seq, nuclei are clustered computationally based on the similarity of their gene expression profiles, enabling the deduction of cluster-averaged transcriptomes that represent different cell types.

As *Drosophila melanogaster* is a leading model organism it is used as a reference genome to map orthologues between species, annotate potential cell types, and understand gene expression profiles. Renal samples include those from the Malpighian tubules, the insect equivalent of the mammalian kidney, and the cryptonephridial complex, a powerful water-extraction system commonly found in, though not exclusive to, beetles.

In *Drosophila***,** the Malpighian tubules divide their function between two major cell types: the larger principal cells, which house cation transporters, and the smaller stellate cells, which are associated with chloride and water channels. There is evidence suggesting that this two-cell model may apply to the Malpighian tubules in other Dipterans (flies), in which stellate cells are documented. However, little is known about how well this system is conserved in other insects.

In this project, we have analysed data and compared species across several insect groups including flies (Diptera), moths (Lepidoptera), beetles (Coleoptera), bees (Hymenoptera) and locust (Orthoptera), with the aim of discovering if indeed two cells are better than one for insect renal function and whether this organisation appears throughout the various insect orders and renal tissues.

921S **The Ommochrome pigmentation pathway: a case study for using metabolomics with transcriptomics** Sue A Krause[1], Karen McLuskey[1], Joe Wandy[2], Rónán Daly[3], Karl Burgess[4], Shireen Davies[2], Julian Dow[2] [1]School of Molecular Biosciences, University of Glasgow, [2]University of Glasgow, [3]MVLS-SRF, University of Glasgow, [4]University of Edinburgh

In any given tissue, the metabolome encompasses all the compounds generated through the metabolic processes within its cells. Integrating metabolomic data with insights from the corresponding transcriptome provides a more comprehensive understanding of the broader metabolomic landscape. Here we use such a multi-omics approach to uncover details of how the Malpighian tubules are involved in both processing and storage of molecules found in the ommochrome pigmentation pathway.

Drosophila eye color is determined by two distinct pigmentation pathways: the ommochrome pathway, which produces a brown pigment, and the pteridine pathway, which produces a red pigment. Together, they give rise to the characteristic reddish-brown eye color observed in the fly.

By combining data from FlyMet (a tissue-specific metabolome web application) with FlyAtlas2 (a tissue-specific transcriptome database), we can identify the tissues that synthesize, transport, and store xanthommatin, the ommochrome eye pigment, and its precursors. The metabolomic data indicates which tissues store the metabolites, while the transcriptomic data reveals which tissues express the genes/enzymes that are actively synthesizing them.

We find the metabolites of the initial steps of ommochrome pathway are synthesized in the fat body and transported via the hemolymph to be stored in the Malpighian tubules of adult and third instar larvae *Drosophila.* In addition, absorption spectra is used to support the metabolomic data.

By integrating transcriptomic data with metabolomic findings, we gain a detailed view of the dynamic movement and storage of Ommochrome pathway metabolites, highlighting the interplay between metabolic synthesis, transport, and storage in different tissues.

922S **Transgenic fluorescent tools at the Bloomington Drosophila Stock Center** Xiangzhong Zheng Department of Biology, Indiana University

Fluorescent probes have been widely used to report gene expression patterns and track physiological activities. Sophisticated modifications of fluorescent proteins make them suitable for visualization of subcellular processes, detection of dynamic intracellular activities and intercellular communications. The Bloomington Drosophila Stock Center (BDSC) distributes a large number of transgenic florescent reporters and regulators that can be used to label cellular organelle, trace lineage, detect synaptic connections, monitor signal transduction and physiological changes, manipulate protein localization and control neuronal activities. This poster will help users select relevant fluorescent transgenic stocks on the BDSC website for various research interests.

923T **Ecdysone Signaling and Lipid Metabolism in Tuberous Sclerosis Complex (TSC) through Oenocyte Regulation** Kerui Huang[1], Norbert Perrimon[2,3] [1]Genetics, Harvard Medical School, [2]Blavatnik Institute, [3]Howard Hughes Medical Institute

Tuberous Sclerosis Complex (TSC) is an autosomal dominant disorder caused by loss-of-function mutations in TSC1 or TSC2 genes, affecting approximately 2 million people worldwide. The TSC1-TSC2 complex is a crucial regulator of the mechanistic Target of Rapamycin (mTOR) pathway, controlling cell growth, proliferation, autophagy, and lipid synthesis. Current therapies, including rapamycin, offer only cytostatic effects, with tumors often regrowing upon treatment cessation. This underscores the need for novel therapeutic targets within the mTOR regulatory network. We hypothesize that steroid signaling, particularly through ecdysone, may play a key role in lipid regulation in TSC pathology.

Our previous work in *Drosophila* revealed that muscle-derived factors, such as PDGF- and VEGF-related factor 1 (Pvf1), modulate lipid synthesis in hepatocyte-like oenocytes via the TOR pathway. We observed that *TSC1* knockdown in oenocytes significantly reduced adipocyte lipid levels, while *TSC1* and *TSC2* overexpression led to lipid accumulation (steatosis) in non-adipose tissues and increased whole-body fat storage. To elucidate underlying mechanisms, we performed single-nucleus RNA sequencing (snRNA-seq) on abdominal tissues containing oenocytes from *Drosophila* with oenocyte-specific *TSC1*,2 overexpression. Motif enrichment analysis identified 81 upregulated genes associated with the ecdysone signaling pathway in oenocytes, implicating the Ecdysone Receptor (*EcR*) as a key transcriptional regulator. Follow-up experiments confirmed that ecdysone transporters, such as *Oatp74D* and *Oatp26F*, are upregulated in *TSC1,2* overexpression models and downregulated upon *TSC1* knockdown. To test the functional significance of ecdysone signaling in lipid regulation, we overexpressed *Oatp74D* in oenocytes, which led to increased triglyceride levels. Furthermore, simultaneous knockdown of *EcR* in *TSC1*,2-overexpressing oenocytes reduced steatosis and whole-body triglyceride accumulation. These findings suggest that *TSC* modulates lipid metabolism via ecdysone signaling, likely influencing steroid hormone accessibility in peripheral tissues. Our results highlight a novel role of ecdysone signaling in TSC-associated lipid dysregulation, positioning ecdysone transport and receptor pathways as potential therapeutic targets for TSC. This work offers new insights into lipid metabolism in TSC and suggests future directions for steroid-based or combinatorial therapies that could augment the effectiveness of mTOR inhibitors.

924T **Nazo, the Drosophila homolog of the NBIA-mutated protein – c19orf12, is an ER associated protein required for triglyceride homeostasis** Sreejith Perinthottathil[1,2], Rajnish Bharadwaj[2], Kristen Patten[2] [1]Eastern New Mexico University, [2]University of Rochester Medical Center

Lipid dyshomeostasis has been implicated in a variety of diseases ranging from obesity to neurodegenerative disorders such as NBIA. Here, we uncover the physiological role of Nazo, the Drosophila homolog of the NBIA-mutated protein – c19orf12, whose function has been elusive. Ablation of Drosophila c19orf12 homologs leads to dysregulation of multiple lipid metabolism genes. nazo mutants exhibit markedly reduced gut lipid droplet and whole-body triglyceride contents. Consequently, they are sensitive to starvation and oxidative stress. Nazo localizes to ER-lipid droplet contact sites and is required for maintaining normal levels of Perilipin2, an inhibitor of the lipase – Brummer. Concurrent knockdown of Brummer or overexpression of Perilipin2 rescues the nazo phenotype, suggesting that this defect may arise from diminished Perilipin2 on lipid droplets leading to aberrant Brummer-mediated lipolysis. Our findings provide novel insights into the role of c19orf12 as a possible link between lipid dyshomeostasis and neurodegeneration, particularly in the context of NBIA.

925T **Delayed developmental time prolongs lifespan through inactivation of developmental STING-NF-κB signaling in Drosophila** Ping Kang[1], Hua Bai[2] [1]Genetics, Development and Cell Biology, Iowa State University, [2]Iowa State University

Developmental time (or time to maturity) is strongly associated with an animal's maximum lifespan. Individuals who mature late often live longer. However, the genetic mechanisms underlying this phenomenon are largely unknown. This is mainly because most of the longevity genes identified previously are the regulators for growth rate, but not developmental time. To address this knowledge gap, we genetically manipulate prothoracicotropic hormone (PTTH), the major regulator of developmental timing in Drosophila, to dissect the genetic mechanisms linking developmental time to longevity. Loss of PTTH results in delayed developmental timing without altering the growth rate. Intriguingly, we find that PTTH mutants are long-lived despite their larger body size. Mechanistically, we show that loss of PTTH blunts age-dependent chronic inflammation, specifically in fly hepatocytes ("oenocytes"). Besides its role in ecdysteroid biosynthesis, PTTH also regulates many novel metabolic and developmental processes during the larva-to-adult transition. Unexpectedly, we show that PTTH mutants exhibit reduced Sting-NF-κB signaling during fly development. Time-restricted and tissue-specific silencing of either Sting or NF-κB/Relish in oenocytes during early pupal stages significantly prolongs adult lifespan. Our studies establish a new aging model that uncouples developmental time from growth rate and unveil Sting-NF-κB signaling as a novel developmental program in determining adult lifespan.

926T **Investigating the role of miRNAs in sensory organ specification and Notch signaling** Rebeccah Stewart[1], Diane Bortolamiol-Becet[1], Fernando Bejarano[2], Hong Duan[2], Eric Lai[1] [1]Developmental Biology, Memorial Sloan Kettering Cancer Center, [2]Memorial Sloan Kettering Cancer Center

microRNAs (miRNAs) are ~21-24 nt RNAs that regulate gene expression, development, and physiology, and the subject of this year's Nobel Prize. However, a central conundrum remains in the field. While individual animal miRNAs often have hundreds of conserved targets, the majority of animal miRNA knockouts exhibit subtle or incompletely penetrant phenotypes. This has fostered the notion that miRNAs mediate "fine-tuning", or have roles that are compensated for by other regulatory means to maintain biological robustness. To gain further insights, we utilize Drosophila genetic tools for largescale in vivo assays that are not feasible in mammalian systems. Here, we report a genomewide screen of UAS-miRNA transgenes for phenotypes in the notum. Using several Gal4 drivers, we recovered numerous miRNAs that perturb notum fusion, epithelial polarity, cell survival or growth, and/or bristle patterning. We focused on defects in the mechanosensory bristle organs. Each of these originates from a sensory organ precursor that undergoes a series of asymmetric divisions to yield several specialized support cells and a neuron. Some miRNA hits yield supernumerary bristles, reflecting ectopic proneural protein activity or reduced Notch signaling. We also found miRNAs that perturb asymmetric divisions within the bristle organ, implying modulation of Notch activity. In support of this, we found that several miRNA-induced phenotypes were modified in sensitized backgrounds with reduced or elevated Notch signaling. Finally, we used genetic assays and reporter tests to connect several miRNA hits to specific target genes in the Notch pathway. In ongoing work, we are investigating which miRNAs are normally expressed in sensory lineages, using single cell sequencing and transcriptional reporters, and investigating corresponding miRNA deletions. This work outlines a genetic strategy to elucidate in vivo miRNA activities and to link them to biologically relevant targets. While this may yield insights to their endogenous functions, another salient conclusion is that miRNA dysregulation, particularly in concert with major signaling pathways, has broad potential to elicit disease and pathogenic conditions.

927F **Ligand-independent Notch signaling actively maintains intervein fate during wing vein patterning in *Drosophila*** Julio J Miranda-Alban[1], Zachary L Baker[2], Carol D Dilts[1], Ilaria Rebay[1,2] [1]Committee on Development, Regeneration and Stem Cell Biology, University of Chicago, [2]The Ben May Department for Cancer Research, University of Chicago

The reliable acquisition of cell fates in precisely organized spatiotemporal patterns is a fundamental feature of metazoan development. Tissue patterning requires the tightly regulated formation of distinct multicellular domains and well-defined separations between them, as seen in the stereotypical vein pattern of the *Drosophila* wing. In this system, the accurate specification of veins and intervein regions, along with the establishment of clear boundaries between them, relies on the coordinated deployment of cell signaling mechanisms. As the early pupal wing elongates proximo-distally, and its dorsal and ventral epithelia appose, EGFR and BMP signaling promote the formation of broad vein regions that initially lack clear borders. Developing vein cells, which express the ligand Delta, activate Notch receptor signaling in flanking cells to repress vein fate, thereby refining vein thickness and delineating distinct vein-intervein boundaries. Notably, upon disruption of Notch activity, we observed the appearance of ectopic vein structures in the center of the large intervein areas. This hinted that Notch-dependent active repression of vein formation might not only be required in cells immediately adjacent to the endogenous veins, but also deeper within intervein domains.

In this study we have combined a sensitive signaling reporter with genetic mosaic approaches to investigate the requirement for Notch signaling in intervein areas distant from vein-intervein borders. Examination of superfolder GFP driven by a *Notch responsive element*, NRE>sfGFP, suggests that low levels of Notch signaling are widespread across the pupal wing epithelium. Clonal analysis confirms that Notch-mediated vein repression is actively required within intervein regions, including cells located far from a major ligand source. Consistent with this, while Delta is the primary driver of Notch signaling near the veins, we find that Notch activity is sustained within intervein domains in a Delta/Serrate-independent manner. To our knowledge, this is the first report of a developmental role for ligand-independent Notch activation in non-circulating cells. More broadly, our findings suggest that these low levels of ligand-independent Notch activity may not only maintain intervein fate, but may also prime these cells to engage Delta-Notch feedback mechanisms that counteract excessive vein formation, thereby thoroughly preserving the integrity of vein-intervein patterning.

928F **The evolutionarily conserved EHMT1/G9a histone methyltransferase family regulates sleep maintenance through ROS homeostasis in insulin-producing cells** Mireia Coll-Tané[1], Lara V van Renssen[1], Boyd van Reijmersdal[1], Jie Han[1], Franziska Kampshoff[1], Chiara Pignato[1], Rik Scharn[1], Human Riahi[1], Nahiua N Gong[2], Matthew S Kayser[2], Marieke Klein[1], Tjitske Kleefstra[1], Annette Schenck[1] [1]Department of Human Genetics, Radboud University Medical Center, [2]Departments of Psychiatry and Neuroscience, Chronobiology and Sleep Institute, Perelman School of Medicine at the University of Pennsylvania

Kleefstra syndrome (KS), a neurodevelopmental disorder caused by loss-of-function mutations in the chromatin remodeler *EHMT1*, is characterized by intellectual disability, autism, and devastating regressive episodes marked by irreversible loss of adaptive skills. Severe sleep disturbances are the only early indicator of these regressive periods, and they may exacerbate the negative outcomes of these episodes. However, the occurrence and impact of sleep disturbances in KS remain poorly understood, and effective interventions are lacking. In this cross-species study, we characterized the function of the evolutionarily conserved *EHMT1*/*G9a* gene family in sleep. We found that 82% of KS individuals show sleep maintenance insomnia, specifically sleep fragmentation with frequent nighttime awakenings. Additionally, common variants in *EHMT1* are associated with short sleep and frequent insomnia symptoms, demonstrating that EHMT1 impacts sleep in KS as well as in the general population. *Drosophila G9a* mutants recapitulate sleep fragmentation seen in humans with mutations and common variants in *EHMT1*. Investigating the cellular origin of sleep fragmentation in *Drosophila*, we found that knockdown in either insulin-producing cells or the fat body is sufficient to induce these. Moreover, G9a is also required in these metabolic tissues for learning. Metabolomics analysis of *G9a* mutants revealed a broad metabolic deregulation, particularly in the methionine metabolism pathway; *G9a* mutants show a significant increase in methionine sulfoxide (Met-SO) levels, the product of methionine oxidation by reactive oxygen species (ROS). Knockdown of the enzyme reducing Met-SO, *MsrA*–a G9a transcriptional target–in insulin-producing cells (but not fat body) recapitulates the sleep disturbances of *G9a* mutants. Strikingly, developmental but not acute treatment with the antioxidant n-acetylcysteine-amide fully rescues the sleep fragmentation present in *G9a* mutants, indicating that G9a regulates sleep integrity via ROS homeostasis. Finally, we demonstrate that a translational behavioral regime based on human sleep-restriction therapy can override the developmentally-installed defects, rescuing sleep fragmentation in adult G9a models. Our work provides novel insights into the epigenetic control of sleep, the pathophysiology of metabolic and sleep disturbances in Kleefstra syndrome, and new targets and approaches for their treatment.

929F **Quantitative analysis of expression and function of Piezo and SERCA in *Drosophila melanogaster*** David Gazzo[1], Jeremiah Zartman[2] [1]University of Notre Dame, [2]Chemical and Biomolecular Engineering, University of Notre Dame

Mechanosensitive ion channels and calcium (Ca^{2+}) signaling regulators are crucial for maintaining cellular and tissue homeostasis, yet their interconnected roles in organogenesis and disease remain incompletely understood. For example, Piezo channels, key mechanotransducers, and SERCA, a central regulator of intracellular Ca^{2+} dynamics, have been implicated in disorders such as cancer and neurodegeneration. Here, we employed *Drosophila melanogaster* as a model system to investigate the functional consequences of perturbing SERCA and Piezo using live imaging, genetic and pharmacological perturbations, and computational modeling. Piezo expression was assessed spatially in both the larval brain and the wing imaginal disc with a custom-made polyclonal antibody, and SERCA's distribution was also characterized to test for the degree of spatial colocalization. We found distinct spatial and temporal expression patterns of Piezo that contrasted with the uniform distribution of SERCA in the larval brain, suggesting specific roles in Ca^{2+} homeostasis. Here, we also report recent findings from our group: Analysis of expression levels were also complemented with investigations of Piezo's role in regulating epithelial cell topology and bilateral organ symmetry. Notably, Piezo's knockout or knockdown disrupted bilateral symmetry in wing size, underscoring its role in precise organ growth. Computational models demonstrated that Piezo regulates cell proliferation and apoptosis by modulating tension thresholds required for its activation (Mim et al., *Cell Reports*, 2024). Finally, we summarize these new observations considering our recent studies, which reveal Piezo's role in maintaining Ca^{2+} homeostasis, regulating epithelial tissue topology, and contributing to precise organ size control. This study establishes a foundation for further exploration of Piezo and SERCA as therapeutic targets in diseases characterized by disrupted Ca^{2+} signaling and tissue homeostasis.

930F **Restraining Wnt activation and intestinal tumorigenesis by a Rab35 dependent GTPase relay** Tianyu Wang[1,2,3], Siamak Redhai[1,2,3], Kim E Boonekamp[1,2,3], Saskia Reuter[1,2,3], Vaishali Gerwal[1,2,3], Tümay Capraz[4,5], Svenja Leible[1,2,3], Shivohum Bahaguna[1,2,3], Fillip Port[1,2,3], Bojana Pavlović[1,2,3], Michaela Holzem[1,2,3], Roman M Doll[1,2,3], Niklas Rindtorff[1,2,3], Erica Valentini[1,2,3], Barbara Schmitt[1,2,3], Karsten Richter[6], Ulrike Engel[7], Wolfgang Huber[4], Michael Boutros[1,2,3] [1]German Cancer Research Center (DKFZ), [2]Institute of Human Genetics, Medical Faculty Heidelberg, Heidelberg University, [3]Department of Cell and Molecular Biology, Medical Faculty Mannheim & BioQuant, Heidelberg University, [4]European Molecular Biology Laboratory (EMBL), [5]Faculty of Biosciences, Collaboration for joint PhD degree between EMBL and Heidelberg University, [6]Central Unit Electron Microscopy, German Cancer Research Center (DKFZ), [7]Nikon Imaging Center and Centre for Organismal Studies, Heidelberg University

Colorectal cancer (CRC) is a leading cause of cancer-related deaths. Inactivating mutations in *Adenomatous Polyposis Coli* (*Apc*) lead to aberrant Wnt signalling, triggering CRC in about 80% of cases. A key early event in this process is JNK signalling induction in the intestinal epithelium, which promotes tumour growth by enhancing cancer cell survival. However, the subcellular membrane trafficking pathways linking *Apc* loss to JNK signalling remain poorly defined, despite having therapeutic potential. By performing an *in vivo* genetic screen in a fly CRC model, we identified that activation of the Rab35 GTPase reverses aberrant Wnt signalling, while Rab35 inactivation augments this pathway. Inactive Rab35 recruits the Rho GTPase, Cdc42, to the plasma membrane where it becomes active to positively regulate JNK signalling through its effector kinase Slipper. We find that, Cdc42, Slipper and JNK are necessary and sufficient for Wnt signalling in the absence of *Apc* but have no role in Wnt regulation under homeostatic conditions. Utilising single cell RNA-sequencing, we discover a novel GTPase activating protein (GAP), which we named blackbelt, that is upregulated specifically in ISCs when *Apc* is depleted. We show that blackbelt accelerates the hydrolysis of GTP-bound Rab35 and is required for Wnt activation. Our findings provide a mechanistic understanding on how *Apc* depletion triggers a GTPase signalling relay to potentiate the Wnt pathway.

931F **Inter-cell type interactions that control JNK signaling in the *Drosophila* intestine** Peng Zhang, Bruce
Edgar Huntsman Cancer Institute, University of Utah

JNK signaling is a critical regulator of inflammation and regeneration, but how it is controlled in specific tissue contexts remains unclear. Here we show that, in the *Drosophila* intestine, the TNF-type ligand, Eiger (Egr), is expressed exclusively by intestinal stem cells (ISCs) and enteroblasts (EBs), where it is induced by stress and during aging. Egr preferentially activates JNK signaling in a paracrine fashion in differentiated enterocytes (ECs) via its receptor, Grindelwald (Grnd). N-glycosylation genes (*Alg3*, *Alg9*) restrain this activation, and stress-induced downregulation of *Alg3* and *Alg9* correlates with JNK activation, suggesting a regulatory switch. JNK activity in ECs induces expression of the intermembrane protease Rhomboid (Rho), driving secretion of EGFR ligands Keren (Krn) and Spitz (Spi), which in turn activate EGFR signaling in progenitor cells (ISCs and EBs) to stimulate their growth and division, as well as to produce more Egr. This study uncovers an *N*-glycosylation-controlled, paracrine JNK-EGFR-JNK feedforward loop that sustains ISC proliferation during stress-induced gut regeneration.

932S **Transcriptional regulation of mitochondrial functions by Wnt signaling in adipocytes** Mengmeng Liu Tulane University

The Wnt/Wingless signaling pathway is crucial for developmental and metabolic processes in metazoans, with its dysregulation linked to developmental disorders and diseases such as cancer. Our previous studies demonstrated that active Wnt signaling in *Drosophila* larval adipocytes reduces lipid accumulation by promoting lipolysis while inhibiting lipogenesis and fatty acid β-oxidation. Given the central role of mitochondria in energy metabolism and fatty acid β-oxidation, we further investigated the effect of Wnt signaling on mitochondrial function. We observed that active Wnt signaling induces mitochondrial fragmentation, increases reactive oxygen species (ROS) levels, enhances ATP synthesis, promotes mitochondria biogenesis, and triggers mitophagy in larval adipocytes. Additionally, Wnt signaling modulates the expression of genes encoding components of electron-chain transport and factors involved in mitochondrial fission and fusion. Through RNA sequencing and CUT&RUN (Cleavage Under Targets & Release Using Nuclease) assays, we identified a set of Wnt target genes that regulate mitochondrial morphology and function. Together, these findings reveal a direct role of Wnt signaling in regulating mitochondrial function through transcriptional control of mitochondria-related genes in *Drosophila* larval adipocytes.

933S **Tumor growth in Drosophila larval epithelial tissue induces distant organ wasting through fat body metabolic dysregulation** Kewei Yu, Gurpreet Moroak, Esther Verheyen Simon Fraser University

Cancer-associated cachexia is a systemic paraneoplastic syndrome characterised by substantial weight loss due to loss of skeletal muscle and adipose tissue. In the recent decade, Drosophila tumor model studies have elucidated numerous cachexic factors and underlying signalling pathways associated with systemic organ wasting.

We have previously established co-expression of constitutively active Siks with Hipk in larval epithelial discs as a Drosophila tumor model. Larvae containing these epithelial tumors have significantly delayed metamorphosis. This delay is accompanied by an increase in tumor burden and larval volume, transparency of the larval body indicative of loss of fat body, and reduced locomotion due to decreased mitochondria in the larval body wall muscle, all phenotypes reminiscent of cachexia-like syndrome.

Larval development in Drosophila relies on conserved hormonal and metabolic homeostasis, indicating that the significant developmental delay is a result of hormonal imbalance and metabolic dysregulation. The Drosophila fat body is the main site of metabolism and considered analogous to the human liver and fat tissues. Triglyceride and glycogen are mainly synthesized and stored in the fat body, and glucose is taken up and condensed into trehalose in the fat body. We find morphological changes such as rounding and dissociation of fat cells in cachexic larvae, induced by sustained levels of MMP1 potentially degrading DE-Cadherin in the cell-adhesion junctions of the fat body. We also find functional changes in cachexic larval fat bodies such as reduced triacylglycerol levels, indicative of increased lipolysis, and increased trehalose levels.

We will present our work on this novel cachexia model which aims to uncover mechanistic insight to the signaling pathways, metabolic changes and potential secreted factors driving these phenotypes, with the goal of gaining insight into mechanisms of cancer cachexia in humans.

934S **Dietary soft electrophiles upregulate pro-resolving oxylipins in a *Drosophila* model of Parkinson's disease** Swarnali Chatterjee[1], Bianca McCarty[1], Caleb Vandenderg[1], Madison Bever[2], Qiaoli Liang[2], Lukasz Ciesla[1] [1]Biological Sciences, The University of Alabama, [2]Chemistry and Biochemistry, The University of Alabama

As humans age, there is a decline in cognitive abilities due to chronic systemic inflammation, known as inflammaging. This further leads to neurodegeneration and age-related morbidities. Neurodegenerative diseases, like Parkinson's disease (PD), currently have no effective treatment options, emphasizing the need for improved management strategies. Epidemiological data suggest that a regular dietary intake of flavonoids and omega-3 fatty acids enhances cognitive capacity in both animal models and humans and delays the onset of age-related neurological diseases. We propose that dietary phytochemicals like flavonoids may mimic the physiological effects of omega-3-derived pro-resolving and anti-inflammatory molecules. Therefore, they might be involved in the process of the resolution of neuroinflammation. Through bioanalytical approaches, we have identified that specific structural features of these compounds directly contribute to their soft electrophilic and anti-inflammatory properties. Our preliminary results suggest that certain plant-derived soft electrophiles, such as gardenin A, thymoquinone, and vitamin E vitamers, enhance the production of lipid pro-resolving molecules in a paraquat-induced *Drosophila* model of PD. The production of these oxylipins is directly related to an increase in age and the feeding method of the flies. It is seen that *relish* , the human orthologue of NF-kB transcription factor in *Drosophila*, actively participates in the production of pro-resolving lipid mediators. We also employed the MALDI-ToF imaging technique to study the localization and spatial distribution of the pro-resolving lipid mediators in *Drosophila* heads post-paraquat exposure. The images indicate that these phytochemicals modulate the overall lipid profile in the fly heads as well. With one of our broader goals being the analysis of neuroprotective activity of dietary soft electrophiles against PD in the *Drosophila* model, our further research will have a general focus on the pro-inflammatory NF-kB cellular signalling pathway in this model, and the potential to target the Nrf2 factor for novel PD therapies. My future studies will include an insight into the mechanisms by which dietary phytochemicals might bring about pro-resolution effects in this *Drosophila* PD model by participating in post-translational modifications of the proteins involved in NF-kB cellular signalling pathway. In conclusion, our study sheds light on the potential of certain dietary soft electrophiles as neuroprotective agents, which could lead to the development of new preventive and treatment options for neurodegenerative diseases and other age-related chronic disorders.

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