[1] Polarized trafficking of cell-cell adhesion proteins facilitates collective migration during embryonic wound healing

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Collective cell movements drive the formation 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 proteins actin and myosin II assembles around the wound to coordinate cell movements and drive wound closure. In parallel, adherens junction proteins, including E-cadherin, are depleted from former bicellular junctions at the wound edge through endocytosis and accumulate at former tricellular junctions around the wound (wTCJs). 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 the small GTPase Rab11 accumulated around the wound. Thus, we hypothesized that E-cadherin is delivered to wTCJs via endocytic recycling. To examine this further, 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 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.

[2] A Rosette is Not a Rosette is Not a Rosette: A Geometric Model for Deconstructing Axis Elongation Kinetics

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A key challenge in developmental morphogenesis is understanding how collective cell behaviors drive tissue deformations. Of particular interest has been the process of axis elongation during early *Drosophila* development, where the embryo's lateral face, known as the germ-band, nearly doubles in length in under 30 minutes. This extension is driven by several cell behaviors, including changes in cell area, aspect ratio, and multicellular rearrangements known as rosettes. Higher-order rosettes involving five or more cells are frequently considered less impactful than four-cell rosettes due to their significantly lower frequency. However, while several models of germ-band extension have been proposed, likely due to their complex dynamics, the contribution of higher-order rosettes to axis elongation remains unresolved.

[3] Dpp and Defective Proventriculus: A Tug-of-War in Determining Eye and Head Fate

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Axial patterning involves defining three key axes—Antero-Posterior (AP), Dorso-Ventral (DV), and Proximo-Distal (PD)—essential for the transition from a monolayer organ primordium to a three-dimensional structure. 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.

[4] Control of Crag's localization and activity in the polarized deposition of basement membrane proteins in epithelial cells

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Apical-basolateral polarity (ABP) is essential for proper functioning and structural integrity of epithelial cells, which are crucial to organismal development. The basement membrane (BM), a specialized extracellular matrix underlying the epithelial cells on their basal side, contributes to ABP establishment and maintenance by providing vital environmental cues. Despite its importance, the mechanisms that confine the BM to the basal side are poorly understood. To study the BM deposition, we use the follicular epithelium (FE) of the *Drosophila melanogaster* ovary as our model system. Using the FE, the GEF/RabGTPase complex, Crag/Rab10, has been shown to be a key regulator of the biological pathway specifically dedicated to the basal restriction of BM components. However, the exact mechanism governing Crag's polarized intracellular localization and its function in BM polarity remains yet to be elucidated. Crag, a multidomain protein, contains the N-terminal DENN domains responsible for GEF activity and a Calmodulin Binding Site (CBS) responsible for Calmodulin-binding activity. Our structure-function analysis of Crag reveals that CBS has a critical role in Crag's localization, while the DENN domains are necessary, but not sufficient, to restrict the BM components basally. Additionally, we also show that Crag not only controls the activity of Rab10, but also Rab 8 – another key regulator of BM polarity. This suggests a more central role of Crag in the pathway regulating BM polarity. Altogether, our findings enhance our understanding of Crag's role in BM polarity and provide insights into the molecular mechanisms underlying the restriction of the BM.

[5] Condensin upregulation is associated with Crohn's Disease and drives cellular senescence, cell death and intestinal permeability in *Drosophila melanogaster*

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Principal Investigator

Crohn's Disease (CD) is a type of Inflammatory Bowel Disease involving chronic, relapsing inflammation in the gastrointestinal tract. The etiology of CD is multifactorial and includes intestinal epithelial cell dysfunction and intestinal barrier breakdown. However, the underlying molecular mechanisms that increase intestinal epithelial barrier permeability in CD patients remain elusive. We recently discovered that transcript and protein levels of condensin subunits are significantly elevated in CD patient ileal epithelial cells. Condensins are multi-subunit complexes known for promoting mitotic chromosome condensation and for organizing chromatin throughout the cell cycle. Our data demonstrate that overexpression of condensin II subunit, NCAPD3, in intestinal epithelial cell lines rearranges heterochromatin into senescence associated heterochromatin foci (SAHFs), and promotes cellular senescence and death. *In vivo*, overexpression of the NCAPD3 homolog, CAP-D3, in the *Drosophila* midgut (similar to the mammalian small intestine), also increases senescence and death through both cell autonomous and non-cell autonomous pathways. Interestingly, CAP-D3 overexpression in intestinal stem cells promotes intestinal permeability in aged *Drosophila.* Finally, re-analysis of single cell RNA-sequencing experiments performed using small bowel and terminal ileum epithelial cells from Non-IBD or CD patients shows that transcripts of senescent cell markers p16 and p21 are significantly

upregulated in CD patient epithelial cell subtypes which also exhibit significant increases in condensin subunit expression. Together, these data suggest that the upregulation of condensins drives chromatin and cell cycle changes that promote cellular senescence and death of ileal epithelial cells, leading to epithelial barrier defects that may contribute to CD pathogenesis.

[6] Lineage-based dissection of the nervous system organization

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Principal Investigator

Neuronal progenitors give rise to lineally-related groups of neurons, called neuronal lineages. Neurons within lineages tend to adopt similar morphologies and are thought to act in the same or parallel circuits to regulate specific behaviors. By focusing on the development and function of the *Drosophila* ventral nerve cord (VNC), our research leverages the power of the fly model system to dissect the genetic and cellular basis of neural circuit formation and behavior.
We have built a library of lines which enables us to manipulate gene and cell function in any VNC lineage during developmental and adult life. Our initial studies have focused on the 4B lineage. We found that 4B neurons control specific leg movements, those enabling flies to reach their anterior notum, likely for grooming purposes. Ablation of the 4B neurons has no apparent effect on walking behavior but eliminates the fly's ability to groom its back. Optogenetic activation of random neurons within the 4B lineage showed that different neurons control the movement of distinct leg segments, indicating a possible functional organization within the lineage. Using genetic tools, we have divided the 4B neurons into three classes of neurons based on their gene expression and birth order and we are investigating the function of each neuronal class during leg movements via loss and gain of function experiments. The results of these experiments will show whether distinct neuronal classes born at different time windows within a lineage function as modular units to control different aspects of a specific behavior.

[7] Loss of *Fic* causes progressive neurodegeneration in a *Drosophila* model of Hereditary spastic Paraplegia

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Postdoctoral Fellow

Hereditary Spastic Paraplegia (HSP) is a group of rare inherited disorders characterized by progressive weakness and spasticity of the legs. Recent newly discovered biallelic variants in the gene *FICD* were found in patients with a highly similar phenotype to early onset HSP. FICD encodes filamentation induced by cAMP domain protein. FICD is involved in the AMPylation and deAMPylation protein modifications of the endoplasmic reticulum (ER) chaperone BIP, a major constituent of the ER that regulates the unfolded protein response. Although several biochemical properties of FICD have been characterized, the neurological function of FICD and the pathological mechanism underlying HSP are unknown. We established a *Drosophila* model to gain mechanistic understanding of the function of FICD in HSP pathogenesis, and specifically the role of BIP in neuromuscular physiology. Our studies on *Drosophila* Fic null mutants uncovered that loss of *Fic* resulted in locomotor impairment and reduced levels of BIP in the motor neuron circuitry, as well as increased reactive oxygen species (ROS) in the ventral nerve cord of *Fic* null mutants. Finally, feeding *Drosophila* Fic null mutants as well as treatment of patient fibroblasts with chemical chaperone PBA reduced the ROS accumulation. The neuronal phenotypes of *Fic* null mutants recapitulate several clinical features of HSP patients and further reveal cellular patho-mechanisms. By modeling FICD in *Drosophila*, we provide potential targets for intervention for Hereditary Spastic Paraplegia.

[8] Characterization of an OPTN-associated model of ALS in *Drosophila melanogaster*

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Graduate student

Optineurin (OPTN) is a mitophagy adaptor protein whose mutations have been associated with familial forms of Amyotrophic Lateral Sclerosis (ALS). Although much work has characterized the functional consequences of OPTN mutations in cell culture, there are limited in vivo models of OPTN-associated ALS that will allow for a more thorough understanding of the disease pathogenesis. While numerous mutations in 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 model organisms. In our study, we have generated a transgenic fly that expresses a mutated Drosophila OPTN (dOPTN^{E283G}), homologous to the human E478G mutation, for in vivo biochemical and physiological modeling of human ALS. Our preliminary results show that mutant E283G protein aggregates and accumulates in larval motoneurons, consistent with mammalian cell culture studies. Our physiological assessments of the consequences of expressing E283G mutant protein in flies show decreased survival of adult male flies expressing mutant protein compared to WT protein. Mutant male flies also performed significantly worse on locomotor assays, showing more rapid declines in performance with age compared to control flies. We are currently examining potential mitophagy defects in the E283G mutant model and suppression of the phenotypes by overexpressing mitophagy mediators. Overall, this work will provide a valuable in vivo OPTN-associated ALS model for advanced understanding and elucidation of the condition.

[9] A glutamate receptor important for cold sensation in *Drosophila melanogaster*

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Undergraduate student student

Temperature is detected by thermosensory neurons in the periphery, which relay signals to the CNS to elicit behavioral and physiological responses. While heat sensation has been extensively studied, much less is known about cold sensation, mainly due to the lack of technologies that can lower temperatures with high speed and precision. To address this issue, we have engineered devices that can deliver localized cold stimuli efficiently without mechanosensory input. The basic aspects of neurobiology are mostly conserved from flies to mammals, making *Drosophila* a powerful model for neuroscience, including thermosensation. Leveraging the strength of advanced cooling technologies to perform behavioral assays, we found that localized cold stimuli induce profound avoidance responses in *Drosophila* larvae. Different regions of the larval body exhibit distinct behaviors to cold. Optogenetic inhibition of a class of dendritic arborization (da) neurons significantly reduced cold avoidance behaviors in both the larval anterior and midsegment, demonstrating that these neurons are important for cold sensation in *Drosophila*. To identify the cold-sensing receptors expressed in the da neurons, we implemented a candidate gene approach. Surprisingly, genetic mutations and RNAi-mediated knockdowns of a kainate-type glutamate receptor, which is not known as a thermosensor, in the da neurons affected anterior cold responses. Our results suggest that the anterior of *Drosophila* larvae sense and respond to cold through previously uncharacterized neural and genetic pathways, which may be evolutionarily conserved in other organisms. Our study contributes new insights into the mechanisms of cold sensation and provides comprehensive characterizations of acute, cold-evoked behaviors in *Drosophila*.

[10] Loss of Neuropeptide F (NPF) signaling reduces the strength of circadian rest-activity rhythms but not feeding-fasting rhythms

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The circadian system governs crucial physiological and behavioral processes, dictating activities like movement, hormone release, and sleep patterns over a 24-hour cycle. Disruptions to these rhythms can lead to various health problems, including cardiometabolic dysfunction. Research has demonstrated the presence of circadian rhythms of feeding behavior in diverse organisms, but the specific molecules involved are not yet identified. Neuropeptide Y (NPY) influences metabolic activity in mammals, and its counterpart, NPF, affects locomotor activity and feeding in *Drosophila*. Our study aims to investigate whether NPF and its receptor,

NPFR, play a role in regulating circadian rest-activity and feeding-fasting rhythms. We are using behavioral analysis tools, such as the *Drosophila* Activity Monitoring (DAM) and Fly Liquid-Food Interaction Counter (FLIC) systems, to study locomotor activity and feeding behavior in mutant and control flies. Our results indicate that flies lacking functional NPF or NPFR exhibit abnormal rhythms in locomotor activity behavior but not feeding behavior, suggesting a differential disruption in circadian regulation. Understanding these mechanisms will not only shed light on circadian biology in fruit flies but also have implications for addressing circadian rhythm-related health issues in humans.

[11] Lamp1 deficiency differentially affects lipid regulation in larval fat bodies and midgut and causes lipid transport defects

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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.

[12] *Drosophila* Ejaculatory Duct as a model to study post-eclosion growth and post-mitotic polyploid tissue regeneration

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Drosophila Ejaculatory Duct (ED) is secretory tissue of the somatic male reproductive system. The ED is involved in the secretion of seminal fluid components and ED-specific antimicrobial peptides that aid in fertility and the female post-mating response. The ED comprises secretory epithelial cells surrounded by a layer of innervated contractile muscle. The ED grows in young adult males during the first 24h post-eclosion, but the cell cycle status of the ED secretory cells and the role of post-eclosion ED growth have been unexplored. Here, we show that secretory cells of the adult *Drosophila* ED undergo variant cell cycles lacking mitosis called the endocycle, which leads to an increase in the cell and organ size of the ED post-eclosion. The cells largely exit the endocycle by day 3 of adulthood, and growth of the ED ceases, resulting in a tissue containing cells of ploidies ranging from 8C-32C. When endoreplication is compromised in ED secretory cells, it reduces organ size, protein synthesis, and compromises fertility. We provide evidence that the growth and endocycling in young adult male ED is dependent on Juvenile hormone (JH) signaling. We suggest that hormone-induced early adult endocycling is required for optimal fertility and function of the ED tissue. Upon damage, the postmitotic polyploid secretory cells of the ED can compensate, restoring lost tissue mass and morphology by re-entering the cell cycle for compensatory cellular hypertrophy. This compensation also restores fertility, demonstrating it is a postmitotic regenerative mechanism. We propose to use the ED as a novel model to study the regeneration of polyploid postmitotic cells.

[13] Regulation of Spermatogenesis by the Notch signaling pathway

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Notch signaling is crucial in fate acquisition and spatiotemporal patterning. The Notch signaling pathway plays a significant role in gonad development and spermatogenesis, but little is known about the specific targets in the testis. The *Drosophila* testis contains two populations of stem cells: germline stem cells (GSCs) and cyst stem cells (CySCs), 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 the transition zone, where somatic cyst cells progress from early to late cyst cell fate. We have found that increased Notch signaling in somatic cyst cells prevents their complete transition from early to late cyst cell fate, suggesting that Notch signaling is important for this transition. We also found Notch overexpression leads to an arrest in late spermatogenesis and sterility. To explore the downstream effectors of Notch mediating these effects, RNA sequencing was performed on testes overexpressing *Notch* and 407 potential Notch targets were identified. Through RNA sequencing data analysis and immunohistochemistry, we have identified specific members of the *Enhancer of Split Complex* and *groucho*, a homologue of the human *Transducin-like Enhancer of Split* class of genes, as downstream targets of the Notch signaling pathway in spermatogenesis. While other studies have shown that these genes are effectors of Notch signaling, our data shows them as downstream targets of Notch in the testis. We are currently using fly genetics to better characterize the function of these genes in spermatogenesis.

[14] Maternal diet influences embryo development and offspring phenotype in *Drosophila melanogaster*

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Postdoctoral Fellow

Parental diet influences offspring development, and prenatal factors such as the mother's diet and stress can shape the adult phenotypes of their offspring. This aligns with the Developmental Origins of Health and Disease (DOHaD) hypothesis, which suggests that maternal stress and nutrition during pregnancy can impact offspring development and health. In *Drosophila melanogaster*, this phenomenon is particularly intriguing since the developing eggs no longer have contact with the mother after they are laid. This suggests that environmental effects are encoded within the egg itself, influencing the offspring's future development and health. In our study, we exposed female flies to low-, medium-, and high-sugar diets for 10 days before mating them with males raised on a control diet. We assessed multiple phenotypes in both the diet-fed mothers and their F1 offspring, including body weight, lifespan, fecundity, and triglyceride levels (TAG). Preliminary results showed that low-sugar diets reduced maternal lifespan, while high-sugar diets increased TAG levels in mothers. In the F1 offspring, we observed a ~2-hour delay in hatching time in those from high-glucose-fed mothers. Additionally, female offspring from both low- and high-sugar-fed mothers exhibited greater resistance to starvation compared to those from medium-sugar-fed controls. We further analyzed metabolites in embryos from diet-fed mothers using single-embryo RNA sequencing and metabolomic techniques developed in our lab. These findings suggest that maternal nutrition may impose metabolic challenges on offspring, resulting in altered phenotypes, supporting the DOHaD hypothesis even in organisms like *Drosophila* where post-laying contact is absent.

[15] Tyrosine metabolism is required for protecting *Drosophila melanogaster* oogenesis from the negative effects of a high sugar diet

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Staff scientist

Unhealthy diets, obesity, and reduced fertility are associated in *Drosophila* and humans. We previously showed that a high sugar diet, but not obesity, reduces female fertility in *Drosophila melanogaster* due to

increased death of newly formed germline cysts and degeneration of later vitellogenic egg chambers. However, it has remained unclear how genetic factors interact with a high sugar diet. We and others routinely use *Drosophila* carrying mutations in the *yellow* (*y*) and *white* (*w*) genes, which control pigmentation, for investigating the effects of high sugar diets. We therefore tested whether this genetic background influences the response to a high sugar diet. In contrast to *y w* mutants, females lacking *y* and *w* mutations retain normal fertility on a high sugar diet. In addition to controlling body and eye pigment production, *y* and *w* have known roles in tyrosine and tryptophan metabolism, respectively, prompting us to investigate the individual roles of these metabolic pathways in the ovarian response to a high sugar diet. We found that the impairment of tyrosine metabolism is required for the degeneration of vitellogenic egg chambers in response to a high sugar diet. We are currently investigating the potential role of dopamine biosynthesis genes downstream of *y* in sensitizing oogenesis to a high sugar diet and how they might interact with the serotonin pathway downstream of *w* in *y w* mutant females. These studies are broadly relevant to our understanding of how the effects of unhealthy diets might differ depending on genetic factors.

[16] Fluorescent reporters for cellular processes and signal transduction

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Principal Investigator

Transgenic fluorescent proteins have been widely used to report gene expression patterns. Development of fluorescent probes with various modifications enables visualization of subcellular compartments and processes, detection of intracellular physiological activities and intercellular communications. The Bloomington Drosophila Stock Center (BDSC) carries a large number of florescent markers, regulators and reporters that can be used to visualize cellular organelle, trace lineage and synaptic connection, monitor signal transduction events and physiological changes, manipulate protein localization and control neuronal activities. I will talk about how to select and find relevant fluorescent transgenes on the BDSC website for various research interest.

[17] Updates from the *Drosophila* Genomics Resource Center

Arthur Luhur
Drosophila Genomics Resource Center, Indiana University
Principal Investigator

[18] Fly-CURE and Connecting Curriculum: Multi-Institutional Course-Based Undergraduate student Research Experiences in Genetics and Beyond

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Principal Investigator

The Fly-CURE is a multi-institutional course-based undergraduate research experience (CURE) centered on the genetic mapping and characterization of *Drosophila melanogaster* by undergraduate students at 26 institutions (including public, private, community colleges, and minority-serving institutions). To date, undergraduate researchers have successfully mapped and characterized 26 EMS-induced mutants, which has led to local and national scientific presentations by students, as well as eleven peer-reviewed publications with 581 undergraduate co-authors. This project has provided research exposure to greater than 1,500 undergraduate researchers within a classroom setting and student participants report significant gains in their sense of belonging to the scientific community, self-efficacy in research methods, and intent to pursue additional research opportunities. We are expanding the Fly-CURE curriculum through an NSF-funded Research Coordinated Network (RCN) to develop courses in bioinformatics, behavioral genetics, molecular biology/CRISPR, and developmental biology, which can be scaffolded with the genetics Fly-CURE modules or can be implemented as stand-alone CUREs. Through this RCN, we will increase research exposure for students across different courses and provide more opportunities for faculty to incorporate CUREs at their institutions. We are currently recruiting faculty to participate in our RCN. Faculty participants will be provided stipends for curriculum training and implementation, a social network of faculty, a community of like-minded

scientists, continued scholarship opportunities, and support for tenure and promotion.

[19] Single-embryo metabolomics reveals developmental metabolism in the early *Drosophila* embryo
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Postdoctoral Fellow

Early embryonic development is characterized by the transition from maternal factor reliance to zygotic control. These processes set the stage for the embryo's basic structure and cellular differentiation. While relatively detailed knowledge exists of the transcriptional events during early development, little is known about the concurrent metabolic processes. Understanding these processes, however, is important since they are linked to cell fate determination and organ and tissue formation. The primary reasons for the limited progress in the field are technical limitations due to the small amount of material available during early embryonic time windows. Here, we introduce a novel single-embryo methodology that places us in an exciting position to analyze the early embryo's metabolome and transcriptome in an integrated manner and at high temporal resolution. The resulting data allow us to map concomitant metabolic and transcriptional programs in early *Drosophila* embryonic development. Our results reveal that a substantial number of metabolites exhibit dynamic patterns with some changing even before the onset of zygotic transcription. dNTPs for example show a temporal pattern that correlates with cell division patterns in the early embryo. In summary, here we present an operationally simple single-embryo metabolomics methodology and provide a detailed picture of early developmental metabolic processes at unprecedented temporal resolution.

[20] A Low-Cost, Versatile Behavioral System for Sensorimotor and Memory Studies in Head-Fixed *Drosophila*
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Research Staff

Recording neural dynamics and animal actions simultaneously under a microscope is essential for understanding the neural basis underlying behavior. Despite the rapid development of imaging techniques for *Drosophila* neuroscience research, budget-friendly and versatile behavioral systems for head-fixed flies are still lacking. Here, we introduce a novel, affordable system for precise multisensory stimulation and high-resolution behavioral recording in head-fixed flies. The system is compatible with most upright imaging setups, facilitating investigations of the neural correlates in various sensorimotor and olfactory conditioning tasks. Our modular design consists of three main components: (A) a robotic tastants delivery unit, (B) a fly-on-the-ball module, and (C) an odor delivery system. Specifically, the robotic tastants delivery unit costs ~$350, and it delivers agar-dissolved tastants with high spatial (~0.1 mm) and temporal (~100 ms) resolution through an automated XY platform robot. The fly-on-the-ball module, adapted and modified from a previous design, features an upgraded ball-tracking camera (314 Hz) and a pose-tracking camera (76 Hz), coupled with DeepLabCut software. This enables precise, quantitative measurement of various fly behaviors, including locomotion, grooming, proboscis extension, and sleep bouts, costing ~$1,000. Lastly, our four-channel odor delivery system (costing ~$2,500) allows for precise olfactory stimulation and the subsequent study of elicited behaviors in head-fixed flies on the trackball. This versatile system is readily adaptable for other labs and can function as a standalone solution for comprehensive behavioral analysis without imaging. Its affordability and flexibility make it a valuable tool for *Drosophila* neuroscience research and beyond.

[21A] Validating the role of cyclin E in fly models of degeneration
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Apoptosis is an essential process for maintaining tissue homeostasis. Its dysregulation can lead to degeneration and cancer. This process is broadly regulated by the p53 pathway. In a previous study, *Cyclin E* (*cycE*) was found to be a modifier of *p53*-associated eye degeneration in *Drosophila melanogaster*. CycE

works with Cdk2 to control the transition from the G1 growth to the S (DNA synthesis) phase of the cell cycle. It does so by phosphorylating the tumor suppressor protein RB, which in turn regulates the transcriptional regulator E2F. Dysregulated expression of *cycE* is associated with highly aggressive cancers such as triple negative breast cancer (TNBC). TNBC lacks estrogen, progesterone, and human epidermal growth factor receptor 2 (HER2) receptors, making it difficult to treat with conventional hormone therapy. We hypothesize that *cycE* is functioning through the RB/E2F pathway to modify apoptosis and degeneration. In our preliminary studies we have found that altered expression of *cycE* in alternative apoptosis models does not influence degeneration, indicating that it specifically interacts with p53. Future work will also focus on exploring the key biomarkers related to p53 pathway to provide an understanding of its regulatory mechanisms and how Cyclin E contributes to the regulation of cell death.

[22A] Sex-Specific Metabolic Shifts and Altered Enzyme Expression in Nepl15 knock-out *Drosophila*

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Obesity and type 2 diabetes (T2D) are critical global health challenges, with the annual cost of diagnosed diabetes in the United States alone reaching $414 billion in 2023. Both conditions are deeply connected to disruptions in metabolic homeostasis, highlighting the need to understand the molecular mechanisms at play better. Neprilysin, an enzyme known for its role in degrading amyloid-beta and regulating blood pressure, has recently been linked to metabolic regulation, influencing insulin sensitivity and energy balance. However, the precise mechanisms remain unclear. Using the *Drosophila* model, we investigate role of Neprilysin-like 15 (Nepl15) in nutrient homeostasis. The Nepl15 gene is expressed 4.5 times more in adult male flies than in females and has sex-specific effects on nutrient storage. Nepl15 Loss of Function (LoF) mutant males show significantly reduced glycogen and glycerolipid storage, while females display increased glycerolipid levels, despite both sexes consuming similar amounts of food as controls. The expression of key metabolic enzymes, such as Glycogen Synthase, Glycogen Phosphorylase and Fatty Acid Synthase, is altered in Nepl15 LoF flies, with sex-specific changes in metabolite profiles. Female mutants show increased levels of prunin, leucrose, and ribonic acid, while males exhibit elevated levels of fucose, fructose, and linoleic acid. Furthermore, female LoF mutants demonstrate reduced oxidative stress, while males show enhanced mitochondrial membrane potential. These alterations correlate with longer lifespans and increased activity in Nepl15 LoF flies. Our findings provide insights into the molecular pathways governed by Nepl15, offering potential therapeutic targets for obesity, T2D, and related metabolic disorders.

[23B] Interaction of Dorsal-Ventral Patterning Selector gene defective proventriculus with Growth Regulatory Hippo Pathway Coactivator *yorkie* in the Developing Eye of *Drosophila melanogaster*

Rohith Basavanahalli Nanjundaiah, Amit Singh, Madhuri Kango-Singh
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Postdoctoral Fellow

The developing eye of *Drosophila* is a well-established model for studying developmental genetic processes and growth regulation. Our long-term goal is to understand the molecular basis of Dorsal-Ventral patterning and growth in the eye by interactions of the dorsal selector genes and growth regulatory genes. We recently identified *defective proventriculus* (*dve*) as a candidate for dorsal-ventral eye patterning that acts as a transcriptional regulator. Gain of function of Dve, results in eye suppression, while loss of function of *dve*, exhibits dramatic eye enlargement phenotypes which raised an interesting question, whether the dorsal patterning gene *dve*, apart from its main function of specifying cells fate, plays a dual role in regulating growth during eye development in *Drosophila*? We hypothesized that Dve may interact with the Hippo growth regulatory pathway to control patterning and growth of the eye. We tested the interactions between Hippo pathway and dorsal-ventral patterning using the GAL4-UAS system and MARCM. To investigate whether *yki* is involved in *dve* domain growth, we used GAL4 drivers in the eye like Dve Gal4 to drive the spatiotemporal expression of transgenes in the dorsal eye, and Ey-Gal4 and GMR-Gal4 to test the epistasis of Dve and Yki before and after MF formation in the larval eye antennal disc for *yki* target genes (*ex, diap1 and hth*) and *dve* target genes mirr, *wingless* (*wg*) (a known and conserved Hippo downstream target) for Dve and Yki mediated effects using reporter assays, clonal analysis and qRT-PCR- based approaches; and our results will be

discussed.

[24B] Multiple mechanisms of action of an extremely painful venom
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Assistant Scientist

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 non-specific, less potent, peptides within the venom. 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.

[25A] Genetic mapping and preliminary identification of the *bang-sensitive 1* gene
Douglas Brusich, Jack Burgess, Esther Oswald, Rachel Faessler
University of Wisconsin-La Crosse, University of Wisconsin-Green Bay
Principal Investigator

The *bang-sensitive 1* (*bas*^{1}) mutant is one of the earliest discovered seizure mutants, originally identified in 1973. The *bas*^{1} mutant exhibits both bang-sensitive and temperature-sensitive seizure behavior. However, identification of the gene responsible for its seizure behavior has never been identified. We used a series of X-chromosome deficiencies for complementation mapping of the *bas*^{1} gene region and assayed by the larval electroshock method. We found the *bas*^{1} phenotype mapped to Df(1)ED7289. Subsequent complementation testing with transposon insertion lines led to preliminary identification of gene *CG9095* as *bas*^{1}. The closest human orthologs of *CG9095* are involved in complement system immune activities, which in humans are linked to febrile seizure and neuroimmune function. Further validation work is necessary to confirm our identification.

[26A] Eyeing the future: Dve’s functional domains and their impact on development and growth
Anuradha V. Chimata, Madhuri Kango-Singh, Amit Singh
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Graduate student

Defective proventriculus, a homeodomain-containing transcription factor, acts as a dorsal fate selector during Drosophila eye development. It is highly conserved across species and its human ortholog SATB1 is highly upregulated in cancer. Mutations in SATB1 cause Den Hoed-de Boer-Voisin syndrome (DHDBV) characterized by developmental delay and craniofacial development defects including hypertelorism. Earlier, we established that *dve* is crucial for determining dorsal fate, which is vital for growth and cell fate decisions. The dve expressing cells (around 150-200) are responsible for inducing the expression of a morphogen *wingless (wg*)/Wnt in the developing eye. These *dve* expressing cells provide an inductive signal that determines eye vs head fate. Recognizing that dve is involved in both growth regulation and cell fate specification, we sought to separate these two independent functions during eye development. To achieve

this, we conducted a structure-function analysis, as dve possesses two isoforms and the Dve protein includes multiple functional domains: the ULD domain, two Hox domains, and a PPP4R2 domain. We hypothesized that these different domains of Dve might regulate distinct functions. In this study, we present our results on the roles of individual functional domains of Dve, as well as combinations of these domains and chimeras with SATB1 in regulation of Wg expression in eye. Insights gained from this research may enhance our understanding of how SATB1 operates in disease contexts, given that it shares similar domains with dve.

[27B] Design of experiments facilitates development of digital twins in systems biology

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Graduate student

A digital twin is a well-informed, predictive, digital representation of a physical system, with multiple industrial applications including energy, transportation, and healthcare. Fruit flies (i.e. *Drosophila melanogaster*) are an effective model organism for studying disease at a small scale due to their low maintenance cost, short lifespans, and many shared genes with humans. Model-based design of experiments (MBDoE), and the creation of a biological digital twin, improves efficiency and productivity of experimentalists, saving time. We leverage nonlinear parameter estimation and MBDoE for developing, validating and optimizing models for fruit fly development, including mechanical (e.g. growth, wing shape) and chemical (e.g. aging, ion channels) properties. Currently, fruit fly digital twins have only captured mechanical movement of the organism, and complex computational models do not have quantified uncertainty against experimental data. Our work will expand on these methodologies through uncertainty quantification followed by efficient experimental design. Laboratory experiments are conducted with fruit flies to generate high-content informative data. The generated data is used to estimate model parameters through parameter estimation. We then use Pyomo.DoE, an open-source, MBDoE package in Python, to evaluate which candidate experiments are optimal with respect to information content. By optimizing the experiments conducted to obtain the necessary information, MBDoE facilitates the production of the final predictive digital model. Ultimately, this will facilitate the translation of digital twins of model organisms into robust methods to study complex human diseases, like neurodegeneration, through the evaluation of homologous genes.

[28B] Drosophila CRC models to study tumor-promoting signaling interactions

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Graduate student

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 APC, likewise promotes cellular proliferation and growth. The Hpo pathway and JNK pathway have also been found to crosstalk extensively 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, *escargot-GAL4* was used alongside the FLP-*FRT* system to drive the expression of dominant-negative *p53*, oncogenic *Ras*, and/or loss-of-function *APC* specifically in intestinal stem cells and at a particular developmental stage. The phenotypes and gene expression patterns of tumor cells were then analyzed via dissection of third-instar larvae and immunohistochemistry. Here, we present preliminary data from these experiments and our progress in developing preclinical models of CRC in *Drosophila*.

[29A] Genetic Screen for Proprioceptor Morphology and Function

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Indiana University
Graduate student

Proprioception is the sensory process of encoding body position. Proprioceptors are the sensory neurons that sense this information. *Drosophila melanogaster* larvae have two proprioceptors in the dorsal region of each hemi-segment of the body wall, termed ddaE and ddaD. The dendrites of ddaE and ddaD have been shown to undergo deformations as the peristaltic muscle contractions travel along the longitudinal axis of the body during crawling. How these neurons sense and respond to the dendritic deformations is largely unknown. Mutations in *Drosophila transmembrane channel like (tmc)* prevent Ca^{2+} responses of these cells during movement, indicating that TMC is required neuronal activation during crawling. Notably, ddaE responds robustly to forward crawling and not reverse crawling, while ddaD responds to reverse crawling but not forward. This directional selectivity is a common feature of TMC expressing cells. From mammalian studies, we know TMC relies on additional factors for its directionally selective activation. We are testing the hypothesis that TMC in proprioceptors may also depend on additional co-factors such as those that are important in mammalian hearing. 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.

[30A] Clevidipine to the rescue: A potential treatment for LMNA-associated muscular dystrophy
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Undergraduate student

The *LMNA* gene encodes lamins, intermediate filament proteins that make up a meshwork lining the inner side of the nuclear envelope. Mutations in the *LMNA* gene cause a group of diseases known as laminopathies. These include three rare types of muscular dystrophy, collectively called *LMNA*-MD. Mutations resulting in amino acid substitutions that affect all three domains of lamins (N-terminal head, a coiled-coil rod, and a C-terminal Ig fold-like) cause *LMNA*-MD. Currently, there are no treatments for *LMNA*-MD other than symptom management. We have generated Drosophila models of *LMNA*-MD that recapitulate aspects of the human disease. In these models, we express mutant *Lamin C* (orthologue of *LMNA*) in the larval body wall muscles using the Gal4/UAS system. These larvae have reduced motility and die at the pupal stage. Using these models, we have performed and unbiased drug screen to identify compounds that rescue lethality. Larvae with muscle-specific expression of Lamin C R264 (rod domain affected) ingested fly food containing drugs throughout development. Vials were scored for dead pupae and live adults. Clevidipine, an L-type voltage gated calcium channel blocker partially rescued lethality. To test for broad efficacy, we are currently feeding clevidipine to larvae possessing muscle-specific expression of *Lamin C* R264W (rod domain affected), and H545P, M553R, and R564P (Ig-fold domain affected). Our findings will provide insights on disease mechanisms and identify a potential treatment for individuals with *LMNA-MD*.

[31B] Investigating Gene Regulatory Networks in Somatosensory-Processing Neurons
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Undergraduate student

Sibling neurons acquire distinct cell fates depending on the timing of their birth from a common progenitor. This fate diversification is governed by gene regulatory networks, with transcription factors orchestrating the precise expression of genes that determine neuronal identity. However, understanding how birth timing dictates neuronal fate is difficult due to limited cell-specific reagents and tractable model systems. In *Drosophila melanogaster*, Even-Skipped Lateral (EL) neurons offer an excellent model for studying temporal cell fate specification as they are labeled by the conserved homeodomain transcription factor, Even-skipped (Eve), and differentiate into mechanosensitive (early-born) or proprioceptive (late-born) neurons depending on their birth timing from neuroblast 3-3 (NB3-3). Eve is required for both fates, suggesting it serves as a

common determinant. Recent unpublished data reveals that the helix-loop-helix transcription factor Collier/Knot is expressed in all EL neurons but is only necessary for Eve expression in late-born ELs, indicating distinct gene regulatory networks in early- and late-born neurons. To investigate the regulatory inputs promoting Eve expression, we employed a transcription factor motif scanning algorithm to predict six factors that could bind to the *eve* enhancer. We generated single-gene mutants for these factors and assessed their effects on Eve expression. Surprisingly, none of the predicted factors caused a significant reduction in Eve expression in either early- or late-born ELs, suggesting the existence of a more complex regulatory network. Future research will explore the potential combinatorial effects of these factors, offering insights into mechanisms of neural diversity and cell fate specification.

[32B] Qualitative and Quantitative Evaluation of the Differences between Indy and Indy-2 Protein in Male and Female *Drosophila melanogaster*

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Texas Tech University
Graduate student

I am Not Dead Yet 2 (*Indy-2*) is a gene in Drosophila melanogaster believed to have a similar function to the *I am Not Dead Yet* (*Indy*) gene. *Indy-2* and *Indy* are both protein-coding genes located in the third chromosome. *Indy* has a human ortholog (*SLC13A5*) with critical implications for metabolism, longevity, and overall health. Bioinformatic tools are used to compare the *Indy-2* and *Indy* genes proteins roles' similarities. Bioinformatics database reveals that *Indy* is highly expressed in tissues associated with metabolism whereas *Indy2* shows highest expression in the testis. The multiple sequence alignment result reveals that the *Indy-2* and *Indy* genes protein sequences only share a 43.2% similarity even though both genes' proteins share similar subcellular localization and protein superfamily. The result also shows that *Indy-2* is most expressed in the male testis and not in female ovaries as supported by Gene expression databases when using in situ hybridization techniques which suggests that *Indy-2* is exclusively transcribed in the testis. In contrast, *Indy* is evenly transcribed in organs that are heavily associated with metabolic regulation and cellular respiration in both male and female flies. The analyses result suggest that *Indy-2* protein may have a sex-specific effect and provide parameters for experiments outside a direct comparison to *Indy* when compare expression levels of *Indy-2* in both male and female drosophila flies using qPCR. The comparison involved the *Indy* life-span extension role in w^{1118} wide-type strain versus *Indy-2* mutant strains of male and female flies, in response to starvation.

[33A] Regulation of Rap1 GTPase signaling during collective epithelial migration

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Post-baccalaureate Fellow

Collective cell migration is critical for embryonic development and tissue repair. However, cells can also use the same migration machinery for spreading cancer, resulting in metastasis. To understand the molecular mechanisms that drive collective cell migration, we investigate wound healing in epithelial tissue using the *Drosophila* embryonic epidermis. Wounds are generated by ablating 4-5 epidermal cells in stage 14 embryos using a high-powered laser. The lesions heal rapidly without scarring or inflammation, forming a supracellular actomyosin cable connected by reinforced adherens junctions at the leading edge of the migrating cells. Our previous work has demonstrated a role for the small GTPase Rap1 in coordinating the cell adhesion and cytoskeletal rearrangements required for rapid wound healing. Rap1 GTPase is misregulated in many invasive cancers, and the Rap1 signaling pathway plays a role in epithelial integrity. Thus, understanding the factors that promote Rap1 activity is critical for important questions in human health and disease. Rap1 is known to be regulated by specific GEFs - PDZ-GEF, Epac, and C3G - and a GAP - Rapgap1. RNAi-mediated knockdown of PDZ-GEF had no effects on the rate of embryonic wound closure, cytoskeletal polarization, or cell adhesion remodeling. Visualizing PDZ-GEF using an endogenous GFP tag shows no localization to the wound edge. Together this suggests that other GEFs and GAPs are playing a more important role in activating Rap1 to drive migration. Future work will examine localization and RNAi-mediated knockdown of Epac, C3G, and Rapgap1 to better understand the regulation of Rap1 during collective cell migration.

[34A] Elucidating the Interaction between Ion Channels Piezo and SERCA
David Gazzo, Jeremiah Zartman
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Graduate student

Complex human diseases, such as neurodegeneration and cancer, are triggered by a combination of genetic and environmental stimuli, with disruptions in calcium (Ca^{2+}) signaling being increasingly implicated in their progression. Understanding the pathways regulating Ca^{2+} gradients is critical for developing therapeutic strategies to restore these processes in disease states. Two key calcium channels involved are the Sarcoplasmic/Endoplasmic Reticulum Calcium ATPase (SERCA) and the mechanosensitive ion channel Piezo. Piezo, located in the cell membrane, translates mechanical stimuli into chemical signals by facilitating extracellular Ca^{2+} influx, while SERCA, sequesters Ca^{2+} into internal cellular stores within the endoplasmic reticulum, maintaining cytosolic concentrations and preparing the cell for future signaling events. Together, these channels play a crucial role in controlling Ca^{2+} dynamics. Recently, studies suggest that Piezo and SERCA physically interact, allowing SERCA to modulate Piezo's activity, demonstrating that SERCA can serve as a pharmaceutical target to reduce harmful Ca^{2+} levels in disorders like Parkinson's. However, this interaction remains unexplored, particularly in *Drosophila*, an excellent model organism for studying complex human diseases. To address this gap, we have validated a new antibody targeting SERCA and developed the first *Drosophila*-specific antibody for Piezo. These antibodies have enabled colocalization studies that have revealed their spatial relationship in the wing imaginal disc and larval brain. Furthermore, they will be essential for conducting a proximity ligation assay to definitively confirm their interaction in *Drosophila*, paving the way for broader analyses of their dynamic interplay in *Drosophila* models of Ca^{2+}-related disorders such as those for neurodegeneration and cancer.

[35B] From neurogenesis to oogenesis: Investigating Inscuteable's role in *Drosophila* oocytes
Sahel Ghasemzadeh, Elijah Sidiropoulos, Audrey Garoutte, Dan T. Bergstralh
University of Missouri
Graduate student

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. Our preliminary results show that genetic disruption of *insc* causes egg chambers to halt their growth at approximately stage 5. We are now using a combination of classic *Drosophila* genetic methods and advanced imaging techniques to explore how and why this happens.

[36B] Effect of drop-dead mutation on the integrity of the cortex glial network in *Drosophila* pupal brains
Grace Ghiselli, Edward M. Blumenthal
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Principal Investigator

Proper interactions between neurons and glia are essential for the maintenance of brain integrity, and disruption of these interactions is a hallmark of neurodegenerative disease. In *Drosophila*, neuronal cell bodies physically interact with a single glial subtype, the cortex glia (CG). Individual CGs wrap processes around 50-100 neurons, and together the CGs form a continuous network that spans the entire cortex regions of the brain. The brains of adult flies mutant for the gene *drop-dead* (*drd)* are characterized by morphologically stunted CGs, loss of the contiguous CG network, and profound neuronal apoptosis; these phenotypes are

observed as early as the day of eclosion. The current study sought to determine if, and when, CG network breakdown occurs during metamorphosis. Control and *drd* mutant pupal brains were dissected and the CG network visualized using *wrapper-QF2* and *QUAS-myrtomato* transgenes. The integrity of the CG network was calculated as the percent of DAPI-stained nuclei found outside of the network. Preliminary results indicate that CG network breakdown in *drd* mutants begins between 40 and 80 hours after prepupal formation. These data reveal metamorphosis to be the key developmental stage for the beginning of neurodegeneration in the *drd* mutant.

[37A] The effects of Upd, Ets21c, and mTOR on cell competition in regenerating wing imaginal discs
Jamie Gonzales, Felicity Hsu, Rachel-Smith Bolton
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Undergraduate student

The process of regeneration is driven by many factors to promote the repair and growth of damaged tissues. The factors and networks that influence the process of regeneration remain ambiguous. Regeneration involves a balance of cell death, cell proliferation, and cell competition. Cell competition is the process in which cells with high fitness outcompete low fitness loser cells, causing them to die. Important pathways involved in regulating cell growth and regeneration include the Jak/STAT, JNK, and mTOR signaling pathways. The Upd3 ligand, which activates Jak/STAT signaling, is expressed in the blastema where it promotes regeneration through activation of JAK/STAT. Ets21c, a transcription factor downstream of JNK signaling, is also required for regeneration as it sustains a pro-regenerative gene regulatory network in the blastema. Similarly, mTOR is a regulator of protein synthesis to mediate cell growth and proliferation. These signaling pathways may also affect cell competition. In this study, we aim to further explore the role of cell competition within the regenerating Drosophila wing disc by reducing Upd, Ets21c, and mTOR expression after tissue damage and observing their effects on cell death due to cell competition. By examining the roles that Upd, Ets21c, and mTOR have in cell death and competition, more light can be shed on understanding how these pathways drive tissue regeneration following damage.

[38A] Biological validation of lifespan modeling in *Drosophilla melanogaster*
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Graduate student

Sleep is an evolutionary conserved function, indicating it is necessary for life. Inadequate sleep promotes several disease states that reduce lifespan. The variety of systems affected by inadequate sleep led us to hypothesize a general underlying mechanism exists that is affected by sleep and promotes aging phenotypes. As organisms age, the proteome is no longer efficient at maintaining balance, mitochondrial dysfunction rises, and response to oxidative stress declines. Our lab is using unique mathematical models to predict lifespan in chronologically identical *Drosophila* based on their sleep characteristics. Our predictions successfully binned the flies into two groups, with 30% of the short-lived flies dying between lifespan prediction and experimentation, compared to only 8% of long-lived flies. We tested age-related conditions such as proteostasis, oxidative stress, and energy homeostasis. We found differences in overall soluble protein concentration, with significantly higher concentration in long lived flies ($p<0.05$). Our short-lived predicted flies had a significantly higher GSSG/GSH ratio, indicating oxidative stress, consistent with aging phenotypes ($p<0.0001$). Furthermore, we found reduced levels of AMP in short-lived flies, indicating trouble maintaining the energy required for proteostasis and appropriate oxidative stress response. Our unique modeling allows us to use sleep as the foundation in our search for an underlying general mechanism associated with these adverse health conditions.

[39B] Investigating the role of Lactate dehydrogenase in the intestinal stem cell niche
Kyle Hart, Michael Haydon, Rafael Demarco
University of Louisville
Undergraduate student

Tissue-resident stem cells are required for the constant generation of the specialized cell types present in

tissues and organs. In the fly intestine, intestinal stem cells (ISCs) divide to self-renewal and to give rise to progenitor cells that can eventually differentiate into either absorbative enterocytes (ECs) or secretory enteroendocrine cells (EECs). Over the past several years, many signaling pathways have been implicated in the regulation of ISC maintenance, proliferation and differentiation potential. Though not always the case, some stem cells are thought to rely on glycolysis for energy generation, while their differentiated progeny favors oxidative phosphorylation. However, our initial observations show that EECs express higher levels of the glycolytic enzyme *Lactate dehydrogenase* (*Ldh*) than any other cell type in the intestine, prompting us to investigate the role of this gene in stem cell behavior. Current work focuses on depleting and overexpressing this gene in stem and progenitor cells to understand if and how *Ldh* would affect ISC behavior.

[40B] 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

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Graduate student

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 types of cardiac progenitor cell division: symmetric, asymmetric, and cell divisions at an earlier stage. Additional analysis also revealed a synergistic genetic interaction between *Rx* and *jumu* which indicated that *Rx* and *jumu* functioned through the same genetic pathway to mediate asymmetric cardiac progenitor cell divisions. Finally, we observed that many of these 21 genes and/or their orthologs exhibit genetic or physical interactions among themselves, implying that Jumu is a master regulator that acts as a hub of a cardiac progenitor cell division-mediating network.

[41A] Impact of expression of candidate modifier genes of apoptosis on models of retinal degeneration in *Drosophila*

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The dysregulation of apoptosis, or programmed cell death, is a major factor in a range of disorders such as cancer, neurodegeneration, and cardiovascular disease. Although the major pathways regulating apoptosis are well studied, there is very little known about the impact of genetic variation on this process. Genetic modifiers of apoptosis could present novel therapeutic targets for such disorders. In previous studies, the *Drosophila* Genetic Reference Panel (DGRP) was crossed with three models of retinal degeneration (overexpression of *p53*, *rpr*, or $Rh1^{G69D}$) to identify candidate modifier genes of apoptosis using both genome-wide association analysis and gene expression correlation. In this study, we investigated the impact of varying expression of four of these candidate genes on cell death and degeneration: *PXK* (*CG8726*), *CKIα*, *SPoCK*, and *ELOVL1/7* (*CG31523*). We found that *CKIα*, *SPoCK*, and *ELOVL1/7* (*CG31523*) significantly modified the degree of degeneration in our models, supporting their candidacy as modifiers of apoptosis. We will focus future investigations on *CKIα* and its role as negative regulator of β-catenin, the primary transcriptional effector in the canonical Wnt signaling pathway. We would like to determine how variable expression of *CKIα* affects downstream transcriptional targets of β-catenin as a possible mechanism of modifying apoptotic responses. These results would widen our understanding of *CKIα* as a modifier of cell death as well as its potential as a

therapeutical target for apoptotic disorders.

[42A] Alzheimer's Disease related dysfunction of circadian rhythms
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Undergraduate student

Alzheimer's Disease (AD) remains the most prevalent form of dementia globally. Treatment efforts remain unfruitful, warranting investigations targeting quality of life improvements and slowing of disease progression. Circadian rhythms are daily periodic alterations in body physiology. Circadian dysfunction has been associated with disease states including AD. The nature of the bidirectional relationship between AD progression and circadian dysfunction remains elusive. Our lab used a *Drosophila melanogaster* AD model paired with behavioral, molecular, and morphological approaches to elucidate this relationship's nature. Behavioral analysis of AD model *D. melanogaster* was conducted using a Drosophila Activity Monitoring system. Results exhibited AD *D. melanogaster* depending on external cues for circadian function, with removal of time cues diminishing circadian behavior paired with substantial phase shifting. The function of the core circadian clock in our AD model was assessed using reverse transcription-quantitative polymerase chain reaction analyzing the core clock gene *period*. The core circadian clock functions normally, indicating potential dysfunction downstream of the core clock. These data suggest circadian function is diminished in our AD model. Neurodegeneration was assessed by quantification of the eye area of AD model flies with and without circadian clock function. Data trended towards a significant decrease in eye size for AD model flies with a dysfunctional circadian clock as compared to AD model flies with a functioning circadian clock, but did not reach canonical levels of significance. Further analysis into mechanism may reveal therapeutic targets to improve quality of life and slow disease progression for AD patients.

[43B] *trithorax* (*trx*) and *trithorax group* (*trxG*) gene regulation of cardiac *Hox* gene expression and anterior-posterior patterning of the *Drosophila* heart tube
Sumaiya Islam, Md. Sayeed Abu Rayhan, Adam J. Farmer, Shaad M. Ahmad, and Kristopher R. Schwab
Indiana State University
Graduate student

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 in insects and mammals. However, the precise regulation of cardiac *Hox* expression has yet to be explored in these organisms. Our previous study identified that *trx* is required for the *abd-A* (a *Hox* gene) expression within the posterior embryonic 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. To better define cardiac *trx* and *trxG* activity, we have investigated unique hypomorphic *trx* strains and additional *trxG* mutants for *Hox* expression and patterning within the embryonic heart. Interestingly, most of these alleles in the homozygous state possess normal *abd-A* expression and patterning. We have found that heterozygotes consisting of a hypomorphic *trx* allele in trans to the trx^{E2} null allele phenocopy the *trx* null cardiac phenotype. These preliminary results will enable us to characterize distinct *trx* mechanisms in cardiac gene regulation. Additionally, several *trxG* genes were evaluated for *abd-A* and *Hox* patterning defects. Although cardiac cell division defects were identified in the homozygous mutants of *wds*, *ash1*, and *ash2*, posterior *Abd-A* expression and patterning were normal indicating that *trxG* activity of these genes may be dispensable in cardiac patterning. Overall, these results suggest that *trxG* genes possess unique and diverse functions in regulating heart development.

[44B] Twin roles of the zinc-finger transcription factor Castor: specification of cardiac cell subtypes and regulation of cardiac progenitor cell division
Rajnandani Katariya[1], Abbigayle J. Gamble[1], Brelin Dickerson[1], Andrew J. Kump[1], Melissa Spognardi[2], M. Rezaul Hasan[1], Kuncha Shashidhar[1], Mufazzal Karim Sabbir[1], and Shaad M. Ahmad[1]
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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 *cas* plays 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). Svp-CCs are found in the posterior aorta and the even more posterior heart proper. These regions are determined by the expression of the Hox genes *Ultrabithorax (Ubx)* and *abdominal A (abd-A)*. Intriguingly, both *Ubx* and *abd-A* repress *cas*, and ectopic expression of either of these two Hox genes in the anterior aorta leads to the ectopic specification of Svp- CCs there—a result which phenocopies cas loss-of-function mutants. Collectively, these data raise the possibility that that *Ubx* and *abd-A* specify Svp-CCs in the posterior aorta and the heart proper by repressing *cas* in those regions. In contrast, in the anterior aorta, in the absence of both *Ubx* and *abd-A*, *cas* levels may be sufficiently high to repress the Svp-CC fate. We are presently testing this hypothesis for *cas*-mediated Svp-CC specification and attempting to elucidate the pathways through which *cas* regulates cardiac progenitor cell division.

[45A] The role of the Lithium-inducible SLC6 transporter (List) in lithium toxicity in *Drosophila*
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Principal Investigator

Lithium, a simple alkali metal, is a widely used and effective treatment for mood disorders, with potential applications for other psychiatric and neurological conditions. Despite its clinical significance, the mechanisms underlying lithium's therapeutic benefits and side effects remain largely unknown. To better understand the molecular and cellular basis of lithium's efficacy and toxicity, we use *Drosophila* to study genes that play critical roles in lithium's biological actions. Our transcriptome analysis of adult fly heads identified *CG15088* as being highly upregulated in response to lithium treatment. Given that *CG15088* encodes a putative amino acid transporter in the solute carrier 6 (SLC6) family, we renamed the gene *Lithium-inducible SLC6 transporter* (*List*). Intriguingly, *List* loss-of-function mutants exhibited higher internal lithium levels and dramatically increased mortality following lithium exposure. *List* is primarily expressed in glial cells, the Malpighian tubules, and the hindgut. Notably, *List* knockdown in the Malpighian tubules had the strongest effect on lithium-induced mortality, while glial-specific knockdown also had a significant impact. Metabolomics analysis revealed that *List* mutation and lithium treatment greatly affected redox homeostasis and altered the levels of certain amino acids. These findings demonstrate that *List* provides a valuable entry point for mechanistic studies of lithium's biological effects on physiology and behavior, using *Drosophila* genetics.

[46A] Altered nociception in a *Drosophila* larvae model of Neurofibromatosis type 1
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Research Associate

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.

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.

[47B] Regulation of cell fate gene *engrailed* in late regeneration of *Drosophila melanogaster* wing imaginal discs

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Undergraduate student

The process of regeneration involves the restoration of a damaged tissue back to its original morphology and function. We use the wing imaginal disc of the fruit fly (*Drosophila melanogaster)* as a model to study how tissues can regenerate after damage. One important signaling pathway that is activated after damage is the JNK signaling pathway (c-Jun N-Terminal Kinase). Our lab found that during late regeneration, high JNK signaling can overexpress *en*, which, along with the PRC2 repressor complex member *polyhomeotic*, silences *engrailed* expression. This silencing of *en* expression leads cells with posterior fate to become anterior cells. Buffering agents such as *taranis* and *zelda* have been shown to prevent posterior cell fate disruption by stabilizing En expression. However, it is unclear which regulatory regions of *en* are acted upon by these protective factors as well as by JNK signaling during regeneration. To identify the relevant *en* regulatory regions, we are screening *lacZ* reporter lines that contain parts of the *engrailed* regulatory sequence. We show here that 4 distinct reporter expression patterns are perturbed upon inactivation of Zelda during late regeneration. The results from these experiments will give us further insight into how cell fate genes can be perturbed by JNK signaling after damage, and how protective agents can prevent this perturbation from occurring.

[48B] Nuclear NAD+ synthase NMNAT1 contributes to nuclear atypia and promotes glioma growth

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Glioma is a malignant primary brain tumor with a poor prognosis and short survival. NAD^+ is critical for cancer growth, however, clinical trials targeting NAD^+ biosynthesis had limited success, indicating the need for mechanistic characterization. Nuclear atypia, aberrations in the size and shape of the nucleus, is widely observed in cancer and is often considered a distinctive feature in diagnosis, however, the molecular underpinnings are unclear. We carried out high-resolution immunohistochemical analyses on glioma tissue samples from 19 patients to analyze the expression of NAD^+ synthase nicotinamide mononucleotide adenylyltransferase (NMNAT1), and its correlation with nuclear atypia in gliomas. Utilizing a *Drosophila* model of glial neoplasia, we investigated the genetic role of nuclear NMNAT in glioma growth in vivo, elucidating the cellular mechanisms of NMNAT1 in promoting nuclear atypia and glioma growth. In low-grade glioma and GBM, higher transcription level of NMNAT1 is correlated with poorer disease-free survival. Samples of high-grade gliomas contained a higher percentage of glial cells enriched with NMNAT1 protein. We identified a specific correlation between nuclear NMNAT1 protein level and nuclear atypia. Mechanistic studies in human glioma cell lines and in vivo *Drosophila* model suggest that NMNAT1 disrupts the integrity of the nuclear lamina by altering the distribution of lamin A/C, and promotes glioma growth. Our study uncovers a novel functional connection between the NAD^+ metabolic pathway and glioma growth, reveals the contribution of the NAD^+ biosynthetic enzyme NMNAT1 to nuclear atypia, and underscores the role of nuclear NMNAT1 in exacerbating glioma pathology.

[49A] Testing the role of Discoidin Domain Receptors in Nociception

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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. 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.

[50A] Investigating the relationship between *drop dead* (*drd*) expression in the cardia and peritrophic matrix (PM) formation in *Drosophila melanogaster*

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Undergraduate student

The peritrophic matrix (PM) is a barrier within the *Drosophila* midgut, that is proposed to be important for protecting the gut epithelium from mechanical damage, limiting pathogen entry, and aiding in proper digestion. Disruption of the PM has been linked to changes in gut integrity and food movement, though the full impact of PM absence remains unclear. Mutants in *drop-dead* (*drd*) lack a PM and display additional phenotypes including early lethality, neurodegeneration, and female sterility. This study investigated *drd* expression in the cardia, the site of PM synthesis, in order to isolate gut-specific phenotypes of the *drd* mutant. We screened multiple GAL4 drivers with specific expression patterns in the adult cardia and identified two lines that affect PM synthesis when used to drive *drd* RNAi. The first consistently eliminates the PM and leads to significantly decreased lifespan, while the other shows partial PM loss without any apparent impact on survival. These results suggest a potential link between the *drd* phenotypes of PM loss and adult lethality. Analysis of the spatiotemporal expression pattern of the drivers also suggests that *drd* expression in the cardia is only required around the time of eclosion for continued PM synthesis during adulthood. This research will clarify the contribution of *drd* expression in the gut to the broad array of phenotypes observed in *drd* mutants.

[51B] Investigating the role of Immune cells during *Drosophila* wing imaginal disc regeneration

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Undergraduate student

All organisms face injuries throughout their lifetime, but some species have evolved advanced regenerative abilities to repair or regrow damaged tissues. When tissues are damaged, repair mechanisms activate and trigger the regeneration process. However, how these tissues initiate regenerative processes remain largely unknown. We use a genetic damage inducing system in the *Drosophila* wing imaginal discs, which form adult fly wings, to study damage response and subsequent tissue regeneration. Upon inducing apoptosis in the wing imaginal disc pouch, cell debris emerge and localize, both apically and basally, throughout the tissue. Clearance of these cellular debris is one of the first steps to initiate regeneration. Here, we hypothesize that *Drosophila* immune cells play a critical role in cell debris clearance and, thus, an even more significant role in regeneration. *Drosophila* immune cells are classified into three classes: plasmatocytes, lamellocytes, and crystal cells. Our current data suggests that lamellocytes are predominantly present at cell debris sites early in the regeneration process, prompting further exploration of their role in tissue repair. Additionally, another critical immune cell function is efferocytosis, where apoptotic debris are cleared by engulfment. Using transgenic reporters specific for efferocytosis, we investigate how undamaged epithelial cells in the wing imaginal disc contribute to this process during tissue regeneration. Thus, our findings will provide insights into the cellular mechanisms governing debris clearance and regeneration, with broader implications for understanding damage response processes across species.

[52B] Amino acid starvation during development induces neurotransmitter switching in *Drosophila melanogaster*

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Neurotransmitter (NT) choice is a hallmark of neuronal cell identity. Drosophila has three major neurotransmitters: acetylcholine, glutamate, and GABA. These NTs can be excitatory or inhibitory. NT switching has been observed in many species. NT switching has been shown to play essential roles in behavior and has been implicated in neurodegenerative and neurodevelopmental disorders. However, limitations in current tools have prevented NT switching experiments from being assayed in the entire central nervous system (CNS). Drosophila has over 100,000 neurons in the CNS. We have the tools to assay the entire CNS for NT switches. Using the Gal4-UAS and Flippase mediated recombination systems, our initial assays observed no central NT switch from glutamate to GABA during normal development. Next, we tested whether environmental stressors, starting with the amino acid starvation, could induce NT switch. We starved developing Drosophila larvae on a sugar-only diet during a critical time point when amino acid starvation has the strongest impact on final body size and obtained flies approximately 50% smaller than control siblings. Our preliminary analysis found that starved animals had more neurons with likely NT switches compared to control fed siblings. We are in the process of confirming our findings and investigating the identity of these neurons. In the future, we aim to investigate the molecular mechanism causing the NT switch. We will begin by examining candidate genes in the insulin signaling pathway. We also aim to investigate the effects of other stressful environmental conditions during development to understand NT switching further.

[53A] Identification of the G-protein coupled receptors controlling the basal deposition of basement membrane proteins in epithelial cells

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Undergraduate students

Epithelial cells are a common cell type that are critical for the proper development and physiology of multicellular animals. Epithelial cells also have a unique architecture that is important for their functions, one aspect of this organization is the proper placement of the basement membrane (BM). The BM is a specialized sheet of ECM that lies along the basal side of epithelial cells and is important for epithelial structure, function, and organ morphogenesis. The misplacement of the BM leads to epithelial and development defects and pathologies such as cancer. Our lab studies the processes important for the deposition of BM components using the follicular epithelium (FE) of the *Drosophila* ovaries as a model system. Using the FE, we identified the G-protein coupled receptor (GPCR) signaling component Rcp, as an important regulator of BM deposition. Since Rcp has been shown to facilitate GPCR signaling, we are performing a genetic screen targeting all of the classical GPCRs for their involvement in BM deposition. To screen the GPCRs, we are using RNAi transgenic lines that allow specific knockdown of the different GPCRs in the FE and observe their consequences on the BM using GFP-tagged BM proteins. Using this approach, we have identified one GPCR that has a role in the basal deposition of BM proteins. Altogether, our data will shed light on the components of GPCR signaling that are important for the control of the placement of the BM, a process that is critical for epithelial organization and functions.

[54A] Circadian regulation of and by coactivator complexes mutated in human disease

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Principal Investigator

The biological clock is maintained through a negative feedback loop of transcription factor action, creating a cycle tuned by light and other environmental factors to be approximately one day in length. Here, we examine mechanisms by which transcriptional coactivators that are mutated in human disease may be subject to circadian rhythms and contribute to propagating circadian behaviors. Furthermore, we consider whether disease-causing mutations cause circadian defects before the onset of the related disease.

[55B] *SMAD7* is a modifier gene of LMNA-associated muscular dystrophy and a therapeutic target

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Graduate student

Mutations in *LMNA* cause a collection of diseases known as laminopathies, which include multiple types of muscular dystrophy (*LMNA*-MD). The *LMNA* gene encodes lamins, intermediate filament proteins that line the inner side of the nuclear envelope. *LMNA*-MD is sensitive to genetic background, as individuals with the same *LMNA* mutation can have clinically distinct diagnoses and/or variable disease severity. Here, we describe a family in which four siblings with the same *LMNA* mutation exhibit highly variable muscle disease severity. Using whole genome sequencing, we identified a variant in the *SMAD7* gene, encoding a negative regulator of the SMAD signaling pathway, that segregates with severe disease. Functional tests in Drosophila models of *LMNA*-MD support this variant as an enhancer of muscle disease severity. Expression of the *LMNA*/*LamC* mutation activates the SMAD pathway in muscle, and this activation is enhanced by the *SMAD7/Dad* variant, providing a mechanism for disease enhancement. Furthermore, overexpression of wild type Drosophila *SMAD7/Dad* can reduce SMAD signaling and rescue muscle defects caused by mutant lamins, implicating the SMAD pathway as a therapeutic target. We have identified six additional *SMAD7* variants in the broader *LMNA*-MD population. We are currently testing whether these additional *SMAD7/Dad* variants can enhance muscle defects caused by multiple *LMNA/LamC* mutations, supporting the broad applicability of *SMAD7* as an *LMNA*-MD modifier gene. Collectively, our data represents the first report of an *LMNA*-MD modifier gene that has been functionally tested in a model organism and implicates the SMAD pathway as a therapeutic target for disease treatment.

[56B] Determining the effect of short and long-term ethanol exposure on olfactory preference in *Drosophila melanogaster*

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Around 400 million people - 7% of the world's population - currently live with an alcohol use disorder (AUD.) Modeling AUD in animals like Drosophila melanogaster, fruit flies, is integral for understanding ethanol's impact on the brain and behavior. Most studies to date have exposed flies to acute ethanol exposures – a single sedating dose or short less than 30-minute intoxicating dose, but few studies have examined addiction-associated behaviors after chronic, repeated exposure. Here, we developed an exposure paradigm where flies receive three 10-minute bouts of 50% ethanol vapor spaced by hour rests for one day or one week. After 1-day or 7-day pre-exposure, groups of 100 flies of mixed sex were tested for their ethanol preference using an olfactory trap assay; flies were given 24 hours to make a choice between food with 50% ethanol on it, or water. Since a previous study indicated the possibility of excitotoxity and olfactory neuron cell death after a single high dose of ethanol, we examined if our paradigm caused antennae to blacken. Preliminary results suggest that w- flies show stronger ethanol preference after 1-day and 7-day pre-exposure, but that pre-exposed Caton-s flies show no difference from mock-treated controls. We also saw little to no antennal blackening, suggesting that our paradigm does not hinder flies' ability to smell.

[57A] An in vivo platform to identify pathogenic loci

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Rare genetic disease discovery efforts lead to the identification of new disease genes. PreMIER (Precision Medicine Integrated Experimental Resources) is a collaborative platform designed to facilitate functional evaluation of human genetic variants in model systems. The PreMIER Consortium has evaluated over 50 variants in patients with genetic disorders and developed an *in silico* pipeline to identify potentially pathogenic loci. We used tissue-specific knockdown to screen 20 genes of uncertain significance (GUS) in the fly for six physiological phenotypes including viability, longevity, behavior, motor function, seizures, and neuronal survival. We associated 3 genes with specific physiological processes, arguing the human orthologues may be associated with genetic disorders. Our study highlights the utility of a tissue-specific knockdown platform in *Drosophila* to characterize GUS, which may provide the first gene-phenotype correlations for patients with idiopathic genetic disorders.

[58A] Regulation of proteostasis by sleep in *Drosophila* models of Tauopathy

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Postdoctoral Fellow

Sleep and circadian rhythm dysfunctions are common clinical features of Tauopathies. Increasing evidence suggests that in addition to being a symptom, sleep disturbances can also drive the progression of neurodegeneration. Protein aggregation is a pathological hallmark of Tauopathies, however the molecular pathways behind how sleep affects protein homeostasis remain elusive. Here we demonstrate that sleep modulation influences proteostasis and the progression of neurodegeneration in *Drosophila* models of Tauopathy. We show that sleep deprivation enhanced Tau aggregational toxicity resulting in exacerbated synaptic degeneration. In contrast, sleep induction using gaboxadol led to reduced toxic Tau accumulation in neurons as a result of modulated autophagic flux and enhanced clearance of ubiquitinated Tau, suggesting altered protein processing and clearance that resulted in improved synaptic integrity and function. These findings highlight the complex relationship between sleep and regulation of protein homeostasis, and the neuroprotective potential of sleep-enhancing therapeutics to slow the progression or delay the onset of neurodegeneration.

[59B] Regulation of the competency to generate INPs

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Graduate student

Emerging evidence suggests that intermediate progenitors, stem cell progeny that function to generate differentiated cells, are heterogeneous in developmental and proliferative capacity. The mechanisms allowing stem cells to generate distinct intermediate progenitor subtypes are unknown. We investigate stem cell (neuroblasts) competency to generate distinct intermediate progenitor subtypes (ganglion mother cell [GMC] and intermediate neural progenitor [INP]) in *Drosophila* larval brains. Each asymmetric division of a type I neuroblast generates a GMC that produces two neurons. By contrast, a type II neuroblast always generates an INP that produces 5-6 GMCs. Surprisingly, cell-type-specific enhancers of genes essential for INP generation and function remain accessible in type I neuroblasts suggesting that both neuroblast subtypes are competent to generate INPs. Mis-expressing type I NB-specific transcription factor Asense or its downstream-effector Prospero drives type II NB progeny to bypass an INP identity and directly assume a GMC identity. Removing *asense* or *prospero* function increases the efficiency of induced INP generation by type I NBs. We conclude that the Asense-Prospero transcriptional cascade promotes proper neural patterning by limiting neuroblast competency to generate INPs.

[60B] Disruption of dopamine release from DL1 cluster neurons induces locomotive deficits in *Drosophila* larvae

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Principal Investigator

Dopaminergic neurons regulate diverse neurological processes including movement, reward signaling, executive functioning and memory. Disruption to dopaminergic pathways and DAN cell loss are associated with a variety of human psychiatric and neurodegenerative diseases such as Parkinson's disease. However, the complexity of the dopaminergic system and the diversity of its roles make studying these diseases challenging. *Drosophila* larvae possess a simplified dopaminergic system consisting of only 42 neurons in the anterior brain. Furthermore, larval DANs are known to mediate movement as well as learning and memory, underscoring the conserved nature of dopaminergic systems across taxa. Despite the clear utility of the larval model, the specific dopaminergic neurons involved in modulating locomotion in *Drosophila* larvae remain largely unidentified. We sought to identify the dopaminergic neurons essential for normal locomotion during edge-seeking behavior in *Drosophila* larvae. We employed the Gal4/UAS system to selectively inhibit neurotransmitter release in subsets of dopaminergic neurons using the spider toxin PLTXII and locomotion was assessed using an established gridline assay and a novel Ring maze navigational assay. Our results

show that blocking dopaminergic transmission leads to significant navigational deficits, including excessive reorientation behavior without impairing the central pattern generator for forward peristalsis. By restricting PLTXII expression to key subsets of DANs, we identified the DL1 cluster of dopaminergic neurons as key modulators of locomotion, with loss of function in a single DL1 neuron being sufficient to induce deficits. These findings contribute to understanding how dopaminergic circuits control movement and offer insights into mechanisms underlying neurodegenerative motor disorders.

[61A] The effects of autophagy inhibition and overexpression on the *Drosophila* testis stem cell niche

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University of Louisville

Adult stem cells are required for tissue maintenance throughout lifetime. Within tissues, stem cells reside in microenvironments known as niches, where systemic and local cues are utilized for the control of stem cell behavior. Due to their longevity, stem and niche cells must employ several quality control mechanisms to maintain their number and activity over time. Autophagy is a quality control mechanism that promotes the recycling of damaged cellular components, as well as the breakdown of macromolecules for energy. Within the Drosophila testis stem cell niche, we have previously shown that autophagy is required for the maintenance of somatic cyst stem cells, but dispensable for germline stem cells. However, the role of autophagy in the supporting niche cells (known as hub cells in the testis) remained uninvestigated. Here we show that autophagy is both required and sufficient for the maintenance of hub (niche) cells with age, non-autonomously maintaining the stem cells they support. Using the GAL4/UAS/GAL80^{TS} system, we downregulated the expression of autophagy factors *Atg1* and *Atg8a* specifically within hub cells, resulting in the decrease of both hub and stem cell number. Moreover, overexpression of the autophagy initiation factor *Atg1* within hub cells was sufficient to suppress the age-related decrease in hub and stem cells seen within the testis niche. Together, these results point to a pivotal role for autophagy in the maintenance and function of hub niche cells in the testis. Current studies aim to understand mechanistically the role of autophagy in promoting stem cell niche homeostasis with age.

[62A] Rcp, a regulator of G-protein-coupled receptor signaling, controls the polarized deposition of basement membrane proteins in epithelial cells

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Graduate student

Epithelia are organized in sheets of tightly adherent cells that are polarized along an apical-basal axis. Epithelial cells rely on their cellular organization for their structure and functions. The apical-basal polarity is characterized by the establishment and maintenance of different polarity domains, which rely on the transport and sorting of proteins to their correct locations. A key component of this organization is the basement membrane (BM), a specialized sheet within the extracellular matrix. Its components are produced within the epithelial cells and secreted to the basal side through a dedicated pathway. Despite the BM's important role in epithelial organization, the process of secretion of BM proteins is poorly understood. To study BM deposition, we use the follicular epithelium (FE) of the *Drosophila* ovary as a model system. In a genetic screen looking for new genes involved in the proper placement of BM proteins, we identified a new gene, *Rcp* which is involved in G-protein signaling. The loss of *Rcp* leads to apical mislocalization of BM proteins without primarily affecting the general polarity, indicating that *Rcp* specifically controls the proper placement of BM proteins. Interestingly, *Rcp,* which assumes cytoplasmic and nuclear localizations in the FE, is the first component of a signaling pathway implicated in BM polarity. Finally, we. showed that Rcp genetically interacts with other members of the pathway dedicated to the polarized deposition of BM proteins. Altogether, our data indicate a specific role for *Rcp* in the organization of epithelial organization by regulating the polarized deposition of BM proteins.

[63B] Investigating the effects of a high sucrose diet on the male germline stem cell niche in *Drosophila*

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University of Louisville
Graduate student

Stem cells are crucial for tissue regeneration and homeostasis throughout an organism's life. Tissue-resident stem cells reside in specialized microenvironments, or 'niches,' which signal to regulate stem cell maintenance and function. Previous studies in *Drosophila melanogaster* have shown that metabolic changes can affect stem cell maintenance and function in vivo. Using the male germline stem cell niche as a model, this study investigates the effects of a high sucrose diet on tissue-resident stem cells. We fed *Drosophila* either a regular or high sucrose diet and assessed stem and niche cell populations. Immunofluorescence with cell-specific antibodies was used to quantify stem and niche supporting cells in the testis niche. TUNEL and caspase activity assays monitored cell death, while lineage tracing explored changes in cell fate within the niche. Our findings show that a high sucrose diet significantly reduces the number of niche supporting cells, correlating with a decrease in germline stem cells, indicating a negative impact on niche maintenance and function. Ongoing analyses aim to determine whether this loss of niche cells is due to cell death or conversion to other cell types. This research underscores the critical role of diet-induced metabolic changes in disrupting stem cell niche homeostasis in the *Drosophila* testis and may offer insights into metabolic-related disorders and potential therapeutic strategies.

[64B] "Hippo's Dynamic Duo": How Wg and Yki Orchestrate Tumor Growth?

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Graduate student

Studies with *Drosophila* $Ras^{V12},scrib^{-/-}$ tumor models reveal that Yorkie, a Hippo pathway effector, interacts with other signaling pathways to form a dynamic network in cancer cells. In these models, Wingless (Wg) acts upstream of Caspases, JNK, and Yki, regulating tumor growth and development (Waghmare et al. 2024). We hypothesize that Yorkie is the key downstream regulator of this network. Heterozygosity of *yki* (yki^{B5}/+; $Ras^{V12},scrib^{-/-}$) reduced tumor clone size compared to $Ras^{V12},scrib^{-/-}$ clones, showing effects on survival (low Diap1 expression and change in apoptotic response). Wg is ectopically induced in Ras^{V12} and $Ras^{V12},scrib^{-/-}$ clones. Wg acts upstream of this network, and is also Yorkie's transcriptional target. We tested the interaction between Yki and Wg and found that downregulating Wg signaling ($dTCF^{DN}$; $Ras^{V12},scrib^{-/-}$) also reduced tumor clone size. Downregulating Wg pathway in $Ras^{V12},scrib^{-/-}$ resulted in decreased DIAP1 intensity (survival) when compared to $Ras^{V12},scrib^{-/-}$ tumor clones. To further explore how Wg and Yki promote tumor growth, we created double mutants combining loss of *yki* ($yki^{B5-/-}$) and dominant-negative dTCF to downregulate Wg signaling in $Ras^{V12},scrib^{-/-}$ clones ($dTCF^{DN}$ yki^{B5};$Ras^{V12},scrib^{-/-}$). We have investigated the synergistic effects of double mutants on (a) hallmarks of cancer such as invasion, cell adhesion, and survival signaling by immunohistochemistry-based approach (b) the transcriptional network of $Ras^{V12},scrib^{-/-}$ tumor clones by analyzing the mRNA expression by qRT-PCR and (c) the Hippo pathway (Mst, p-MST), JNK pathway (JNK,p-JNK) and Wg pathway activity by Western blot assays. Here, we present our progress on the organization of the molecular network that involves Wingless and Yorkie.

[65A] Investigating the effects of ethanol exposure on associative memory and light cue preference in *Drosophila melanogaster*

Taneil Ramirez, Emily Petruccelli
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Undergraduate student

Alcohol Use Disorder (AUD) affects millions globally (NIAAA), but it still unclear why some people become addicted. Modeling AUD in animals like *Drosophila melanogaster* (fruit flies) is crucial for understanding ethanol's impact on decision-making. Previous studies have shown that wildtype flies remember and enjoy the experience of intoxication. Here, we investigated whether flies develop memories of a light cue associated with ethanol exposure. Male flies were exposed to 50% ethanol vapor and blue light ('experimental'), water vapor and blue light ('mock-treated'), or never exposed to blue light ('naïve'). Training sessions occurred three times for 10-minutes each day, with 1-hour rests. The day after either 1-day or 7-day training, condition place

preference was assessed for groups of 10 flies in circular locomotion arenas half exposed to blue light and half protected from blue light (darkness). FlyTracker software and R coding was used to track and analyze fly X,Y coordinates. Preliminary results suggest 1-day training caused naïve and experimental, but not mock-treated, to prefer the light cue. Surprisingly, after 7-day training only naïve and mock-treated, but not experimental, flies showed cue preference. We had anticipated that longer training would have enhanced cue preference, not cause a loss of preference altogether. These findings suggest that prolonged exposure may produce more subtle behavioral changes and alter the valuation of ethanol-associated cues. Together this works supports that flies can associate environmental cues with alcohol exposure and that further research can reveal mechanisms underlying AUD etiology in humans.

[66A] Characterization of Phosducin-like Protein 3 in Gametogenesis

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Phosducin-like protein 3 (PhLP3) may function as part of a co-chaperone in the folding of actin and tubulin in the chaperone containing tailless (CCT) complex. Additionally, PhLP3 possesses redox activity in the thioredoxin domain, suggesting redox activity may be important for its function. We have used the *Drosophila* testis as a model to study PhLP3 function, given the importance of actin and tubulin in cellular remodeling during sperm and egg development. The *Drosophila melanogaster* homolog of PhLP3 (PhLP3) is encoded by the *CG4511* gene. Previous studies in the lab demonstrated that PhLP3 is required for spermiogenesis and oogenesis. Male flies homozygous for a P-element insertion in the 5' UTR of *PhLP3* exhibited decreased levels of *PhLP3* mRNA, a failure to elongate the nucleus during spermiogenesis, and infertility. Further study reveals that in the mutant flies, actin-based individualization cones are absent, and microtubule levels may be reduced. We hypothesize that PhLP3 functions to regulate the cytoskeleton during sperm maturation. We are currently using transmission electron microscopy to explore the changes in cell structure resulting from *PhLP3* mutation. PhLP3 also functions in oogenesis, as females are sterile and eggs fail to hatch. Actin filaments and microtubules play a role in localizing mRNAs and proteins to establish polarity in the oocyte. Recent work suggests that *PhLP3* mutant oocytes have reduced Vasa localization to the posterior, suggesting PhLP3 may regulate the cytoskeleton in this context. Our results suggest a critical role for PhLP3 in cytoskeleton-dependent processes during sperm and egg development.

[67B] *Polycomb* (*Pc*) and *Pc Group* (*PcG*) genes repress *trithorax* (*trx*)-mediated *Hox* expression and cardiac patterning within the *Drosophila* heart tube

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The PcG proteins antagonize the activity of Trx Group proteins by repressing the expression of important developmental genes. We have previously identified *trx* as a positive regulator of *Hox* expression and anterior-posterior patterning within the *Drosophila* embryonic heart tube. For example, *trx* maintains the expression of *abdominal-A (abd-A)* in the posterior heart tube and is necessary for heart-proper patterning. Whereas, *Pc* represses *abd-A* expression within the anterior heart tube specifying the aorta. *Pc* regulation of cardiac *Hox* expression has yet to be investigated in either *Drosophila* or mammalian models of heart development. To determine the precise roles for *trx* and *Pc* in *Drosophila* heart development, a *trx, Pc* recombinant strain possessing amorphic alleles was generated to produce homozygous *trx, Pc* null embryos. Remarkably, the *trx, Pc* strain 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, whereas *Pc* activity appears to repress *Hox* activity preventing ectopic expression. The *Pc*-mediated repression of cardiac *Hox* activity implicates the function of the Pc-repressive complex 1 (PRC1), PRC2, and other complexes in cardiac patterning. The investigation of *PRC1* and *PRC2* genes for aberrant cardiac *Hox* expression and patterning has identified *Sex combs on midleg (Scm*) as an PRC1 member responsible for anterior cardiac *abd-A* repression. In contrast, our preliminary screen of the PRC2 genes indicate that these genes are dispensable for *abd-A* repression. These findings identify diverse roles of *PcG* activity regulating cardiac *Hox* activity and

patterning in development.

[68B] Cell reintegration function of the Fasciclin II intracellular domain in the *Drosophila* follicular epithelium

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Epithelia is a prevalent animal tissue; its integrity is key for an organism's normal development. Forming discrete layers, epithelia functions in secretion, absorption, and protection. Complex mechanisms are in place to ensure tissue organization, which can be disrupted by cell division. Cells normally divide within the tissue plane, but a misorientation of the mitotic spindle can result in a daughter cell positioned outside the tissue layer. This cell makes its way home through a process called reintegration. When reintegration fails, "popped-out" cells remain outside the tissue layer. We seek to understand the mechanisms that drive cell reintegration in epithelia. Immunoglobulin cell adhesion molecules reintegrate misplaced cells in our model tissue, the *Drosophila* follicular epithelium. Two such molecules are Fasciclin 2 (Fas2) and Neuroglian (Nrg). Fas2 and Nrg localize along lateral cell borders at immature septate junctions. We understand that Nrg requires a spectrin-based membrane skeleton anchor to function in reintegration and we hypothesize that Fas2 works through a similar mechanism. The Fas2 gene encodes different protein isoforms: some have an intracellular domain, and some do not. Our work investigates which versions are expressed in our studied tissue and if or how their intracellular domains bind other molecules to facilitate reintegration. A yeast two-hybrid screening identified four possible intracellular binding partners, one of which was the known partner Discs Large (Dlg). The other identified candidates, Dystrophin and the uncharacterized protein CG18135, were tested for intracellular interaction with Fas2. Additionally, the Dlg- Fas2 junction was further studied for its role in reintegration.

[69A] Functional analysis of the cariogenic roles of spalt major and spalt-related, *Drosophila* orthologs of human zinc finger transcription factor-encoding genes associated with congenital heart defects

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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. We 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 *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 the contralateral myocardial hemisegments, abnormal curvature of the heart tube, and failure of posterior heart tube closure. 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.

[70A] Exercise Mimetics Rescue Endurance and Climbing Speed in Circadian Mutants

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Circadian rhythm disturbances are associated with various negative health outcomes, including an increasing incidence of chronic diseases with high societal costs. While exercise can protect against the negative effects of rhythm disruption, it is not available to all those impacted by sleep disruptions, in part because sleep disruption itself reduces exercise capacity. Thus, there is a need for therapeutics that bring the benefits of exercise to this population. Our lab has been studying the relationship between exercise and circadian disturbances using a well-established *Drosophila* model of circadian rhythm loss, the Clk^{out} mutant. Clk^{out} mutants have impaired exercise capacity that can be rescued by pharmacological administration of octopamine or by genetic upregulation of the exercise-response genes *sestrin* and *spargel*. We are now exploring the mechanisms behind these rescues by attempting to narrow down which body tissues (e.g. neurons, muscle, fat) are required for *sestrin* and *spargel* to rescue exercise capacity in Clk^{out} mutants, and whether rescue of cycling gene expression is required for these effects.

[71B] Using *Drosophila* denticles as a model system to investigate possible cargos for *ck*/Myosin VIIA during the formation of actin-based protrusions

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Principal Investigator

The formation of actin-based protrusions, such as the denticles in *Drosophila melanogaster*, requires the coordination of extensive actin-associated proteins to crosslink and bundle actin filaments. Mutations in such proteins can cause defects in the shape, structure, and function of actin-based protrusions and provide us with information on the molecular mechanisms of their function. Previous research demonstrates a need for the motor protein *ck*/Myosin VIIA in proper denticle formation, and we hypothesize it transports cargo necessary to build the actin protrusion. Cuticle preps of late-stage embryos from loss of function alleles of *singed, forked*, *shavenoid*, and *ck*/MyoVIIA were examined for their overall denticle morphology as well as for the height, widths, and areas of the denticles. Our data show that mutations to *shavenoid* (sha^{1} and sha^{VA151}) drastically reduce denticle area, while *singed* and *forked* ($sn^{3}f^{36a}$) double mutations cause elongated heights and widened bases compared to both wild type controls and ($sn^{3}f^{36a}$; ck^{13}) triple mutants. Actin staining of *shavenoid* mutant denticles confirm failure of actin organization into bundled structures which are more severe than *ck*/MyosinVIIA mutants. The morphological abnormalities with *singed, forked*, *shavenoid* mutants showcase the genes' importance in proper denticle formation and suggest a role as cargo proteins for *ck*/Myosin VIIA.

[72B] Exploring the Effect of Mutation rates on Lifespan in Fruit Flies

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Errors in DNA replication may lead to increased mutation rate and changes in organism lifespan. Although this assumption may apply to long-lived organisms, how mutation rates affect the lifespan of shorter-lived organisms remains elusive. This project aims to investigate the effect that mutation rates have on the viability and lifespan of short-lived fruit flies. To examine the effect of mutation rate, we introduced well-characterized *pold1* mutations, generating exonuclease and fidelity minus mutant lines. *pold1* encodes a catalytic subunit of DNA polymerase delta, an evolutionarily conserved enzyme complex essential for eukaryotic DNA replication by synthesizing the lagging strands. First, we performed a viability assay with seven *pold1* mutant combinations. From four replicate experiments, we confirmed that all *pold1* mutants had a lowered homozygote viability compared to *wild-type*. Of the crosses, fidelity minus homozygous mutant animals showed the highest lethality, suggesting the importance of proper *pold1* activity during development. Next, we assessed how each *pold1* mutation affects lifespan of the organism. Our preliminary data showed that certain mutants exhibited a reduced lifespan compared to *wild-type* animals. These results suggest that the mutation rate plays a critical role in determining lifespan, even in short-lived organisms.

[73A] Fox transcription factor-mediated morphogenesis of the alary muscles associated with the *Drosophila* heart

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Graduate student

Eight Forkhead box (Fox) transcription factors are essential for proper cardiogenesis in mammals, with mutations in four Fox genes causing congenital heart disease. Our previous work identified conserved roles for 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 describe additional roles of *jumu* and *CHES-1-like* in the morphogenesis of pericardial ligaments, also referred to as alary muscles (AMs) in *Drosophila*. AMs anchor the heart to the embryonic/larval exoskeleton, thereby providing crucial support, stabilizing the heart position, maintaining lumen integrity, and regulating ostia function and heartbeats. Our findings show that mutations in the Fox genes and in *back seat driver* (*bsd*), a *jumu*-activated kinase, exhibit severe AM deformities, truncations, abnormal positioning, and improper myotube attachments. Multiple hypotheses could explain these defects in AM morphogenesis: (1) incorrect specification of AM founder cells, (2) defective myoblast fusion, (3) fusion between myotubes, (4) flawed myotube elongation, (5) incorrectly positioned AM attachment sites due to cardiac progenitor cell division errors, (6) improper muscle attachment site selection, and (7) defective interactions between Fox and Hox genes. Utilizing live imaging and phenotypic and genetic interaction assays, we are attempting to determine which of these hypotheses are correct, and thereby understand the mechanisms by which Fox gene mediate development of associated tissues supporting the heart. The outcomes from this research may shed light on difficult to detect defects in cardiac ligaments and the pericardium, potentially aiding in the prevention of sudden cardiac failure.

[74A] Investigating the Role of SWI/SNF in Posterior Cell Fate Regulation in *Drosophila* Wing Discs

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Drosophila melanogaster has long been a model organism for studying tissue regeneration. In our research, we use a genetic ablation system to induce tissue damage in wing imaginal discs during the third instar larval stage by overexpressing the pro-apoptotic gene *reaper*. The wing imaginal disc of *Drosophila* is divided into distinct regions, with the posterior compartment identity regulated by the expression of *engrailed* gene. High JNK signaling during regeneration can lead to *engrailed* overexpression and eventual silencing, causing posterior cells to adopt anterior fates. Previous studies show that the SWI/SNF chromatin remodeling complex protects these aberrant cell fate changes from happening. Our current research focuses on using a Bap60 RNAi to investigate the role of SWI/SNF in regulating *engrailed* through specific *engrailed* enhancers. We are analyzing *engrailed* enhancer reporters across its regulatory region to monitor changes in expression during late regeneration when SWI/SNF activity has been disrupted. This study will allow us to investigate regeneration specific regulatory regions that can stabilize posterior compartment fate, ultimately identifying a novel mechanism for cell fate regulation during tissue regeneration.

[75B] Stem cell lineages in the *Drosophila melanogaster* ovary require glucose instead of trehalose as a primary sugar source for glycolysis

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Stem cells maintain cell populations in various adult tissues, and there is growing evidence that metabolism regulates these lineages. However, much remains unknown about how metabolic requirements change at specific steps from self-renewal through various stages of differentiation. The germline and follicle stem cell lineages in the *Drosophila* ovary are an ideal model for addressing this question. Unpublished data from our lab showed that glycolysis, but not fatty acid oxidation, is required for specific processes within the germline and follicle stem cell lineages. Trehalose (composed of two glucose subunits) is the predominant circulating sugar in *Drosophila*, while glucose is present at lower levels that fluctuate with diet. However, it remains unclear which of these sugar sources fuels glycolysis in the ovarian stem cell lineages. In this study, we examined the requirements for a major glucose transporter (encoded by *glut1*) and an enzyme that hydrolyses

trehalose into two units of glucose (encoded by *treh*) in these stem cell lineages using FLP/FRT-mediated genetic mosaic analysis. Our results show that glucose is the major sugar source for glycolysis within the germline and follicle stem cell lineages, and that trehalose has a minor role. We are currently expanding our genetic mosaic analysis, and we also plan to utilize RNAi to investigate metabolic requirements in other cell populations in the ovary. Uncovering the specific metabolic requirements at various steps in stem cell lineages will provide a foundation for future research on the mechanisms linking these requirements to proper stem cell self-renewal and progeny differentiation.

[76B] Exploring the Role of miRNAs in Craniofacial Syndromes: A Genome-Wide Approach Using *Drosophila* Models

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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. miRNA serves a vital role in the retina throughout development and in eye diseases. We employed *Drosophila eye* as a model system for genome-wide screening of miRNAs involved in eye defects. We have identified a *miRNA-190* which exhibits strong eye enlargement phenotype. 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 eliminated GFP expression, which confirmed *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. Here, we present a new mechanism of post-transcriptional regulation of *dve/SATB1* expression by miR-190/miR-190a.

[77A] Phenotypic Mapping of *Drosophila* Ventral Nerve Cord Lineages

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The intricate neural connectivity of our nervous system orchestrates a broad variety of behaviors, from simple reflexes to more intricate actions such as playing piano. The Drosophila ventral nerve cord (VNC) serves as an excellent model in exploring the development of neural circuits. The VNC is composed of 34 lineages, which are pools of neurons sharing an embryonic stem cell origin, transcription factor expression, and neurotransmitter usage. By employing a lineage-based approach, we aim to characterize each lineage's contribution to controlling behaviors. By combining CRISPR and trojan exon methods, we have generated a split-GAL4 library recapitulating the expression of lineage-specific transcription factors identified from previously published single cell RNA sequencing data. Our driver lines achieved high specificity targeting individual lineages genetically with minimal off-target expression. These reagents enable instantaneous and precise activation of neurons in specific lineages via optogenetic approaches and the study of behaviors regulated by these lineages. Our findings indicate that each lineage produces distinct behaviors when activated via optogenetic tools such as activated lineage 2A neurons causing a wing buzzing behavior and activated 8B neurons resulting in a jump behavior. By understanding the functional roles of individual lineages, we aim to uncover the underlying mechanisms driving behavior and development including the influences of gene expressions and classifications of subclasses within a lineage. With a foundation of lineage mapping to specific behaviors, we now aim to investigate the genetic basis of neural circuit formation during development by focusing on the function of lineage specific transcription factors.

[78A] The role of retrotransposable elements in neurodevelopment

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Microcephaly is a neurodevelopmental disorder where the head size of the patient is 2-3 standard deviations below average. Recently, we have shown a Drosophila model of microcephaly, which was due to increased retrotransposable element (RTE) activity. In this model, subunits of the condensin complexes were knocked down, mimicking some genetic causes of microcephaly in humans. This model exhibited increased NSPC cell death and increased RTE activity. Excitingly, microcephaly was rescued in the condensin Drosophila model when RTE activity was prevented using Nucleoside Reverse Transcriptase Inhibitiors (NRTIs). While high levels of RTE activity have been associated with several neurodevelopmental disorders, the baseline RTE activity in the brain has been demonstrated to be higher than other tissues. It is currently unclear whether this high baseline RTE activity is required for proper brain development and how RTE activity above this baseline could contribute to neurodevelopmental disorders. This project seeks to understand the function of RTEs during normal brain development and why increased RTE activity can cause microcephaly. Cellular response to DNA damage, likely caused by RTE activity, was determined, at least in part, to contribute to microcephaly in the condensin Drosophila model. Innate immune pathway activation from high RTE expression is also being explored as a mechanism contributing to microcephaly. By understanding the role of RTEs in normal brain development and identifying the mechanisms by which increased RTE activity can cause microcephaly, information on the viability of NRTIs as a treatment for microcephaly, and potentially other neurodevelopmental disorders, will be uncovered.

[79B] *Drosophila* models reveal converging mechanisms of Snyder-Robinson Syndrome and Alzheimer's disease

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Polyamines, including spermidine and spermine, are small, positively charged molecules that are either synthesized by cells or obtained from the external environment. These polyamines interact with negatively charged cellular components—such as nucleic acids, proteins, and lipids—to regulate a wide range of cellular activities. Dysregulated polyamine metabolism has been implicated in several pathological conditions, though the underlying mechanisms remain largely unclear. In this study, we use *Drosophila* models to investigate these mechanisms by comparing alterations in the polyamine pathway between two distinct neurological conditions: Snyder-Robinson Syndrome (SRS), a rare genetic disorder, and Alzheimer's Disease (AD), a common neurodegenerative disorder. SRS is caused by mutations in spermine synthase (SMS), leading to reduced spermine and the accumulation of spermidine. This is accompanied by increased levels of spermidine catabolic byproducts, such as hydrogen peroxide (H_2O_2) and aldehydes, which damage cellular structures, including mitochondria and lysosomes. Inhibiting spermine/spermidine acetyltransferase 1 (SAT1), the rate-limiting enzyme in spermidine catabolism, reduces oxidative stress and restores mitochondrial and lysosomal function in SRS models. In contrast to SRS, SMS and several other genes in the polyamine metabolism pathway are upregulated in AD, leading to the accumulation of spermidine or spermine in different tissues. Interestingly, downregulating SMS in AD models confers protection against the disease by enhancing autophagy. These findings shed light on the complexities of polyamine dysregulation in neurological disorders and offer promising insights for potential therapeutic strategies.

[80B] Metabolomic Profiling Reveals Altered Metabolism in a *Drosophila melanogaster* Model of PLA2G6-Associated Neurodegeneration (PLAN)

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PLA2G6-Associated Neurodegeneration (PLAN) is a rare and progressive neurodegenerative disorder caused by mutations in the PLA2G6 gene, which encodes the calcium-independent phospholipase A2 (*iPLA2-VIA*). These mutations are thought to cause neurodegeneration by disrupting phospholipid metabolism and mitochondrial function. The *Drosophila melanogaster* models of PLAN, which carry loss-of-function mutations

in *iPLA2-VIA* gene, exhibit age- dependent locomotor defects, reduced lifespan, and female-specific fertility issues. Given the role of *iPLA2-VIA* in mitochondria, we hypothesized that age-dependent and sex-specific metabolic abnormalities would be observed in *iPLA2-VIA* mutant flies. Using mass spectrometry, we analyzed small metabolites and lipids from young and aged *iPLA2-VIA* null mutant and control flies, employing SIMCA and MetaboAnalyst software. We identified 195 small metabolites and 379 lipids that passed quality control. In aged male mutants, 27 metabolites were upregulated and 55 downregulated, while 3 lipids were upregulated and 303 downregulated. In aged female mutants, 46 metabolites were upregulated and 36 downregulated, with 130 lipids upregulated and 102 downregulated. PCA revealed close metabolic profile between young iPLA2-VIA mutant and control males, with partial separation in aged flies. In females, distinct separation of metabolic profiles between young and aged flies was observed, with young mutant females resembling aged controls, suggesting premature aging and a possible link to fertility defects. We performed pathway analysis using IPA (Ingenuity Pathway Analysis) software to identify key disrupted pathways. IPA revealed significant disruptions in several key metabolic pathways, including glycolysis, the TCA cycle, and lipid metabolism, highlighting the profound impact of iPLA2-VIA mutations on energy production and lipid processing.

[81A] Biochemical identification of Myosin7A binding partners

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ck/MyosinVIIA is a motor protein that is necessary for the formation of actin-based protrusions such as wing hairs, bristles, and denticles in *Drosophila melanogaster*. *ck*/MyosinVIIA is believed to carry proteins within these structures, however the identities of its cargo proteins are currently unknown. A potential protein binding partner is shavenoid, a protein that interacts with the actin cytoskeleton in bristles and hairs. Loss of function of shavenoid results in small wing hairs and denticles, supporting is role in maintaining the same structures as *ck*/MyosinVIIA. We generated a transgenic fly containing a triple affinity-tagged *ck*/MyosinVIIA tail construct under the control of a UAS-promotor. We co-expressed the triple-tag *ck* tail and GFP-shavenoid with *squash*-GAL4 and performed a protein pulldown for the triple-tag *ck* tail. Current buffer conditions limit the solubility of the *ck* tail construct. Future directions aim to improve solubility of *ck*/MyosinVIIA and test for protein interactions.

[82A] Functional Interrogation of Somatic Mosaicism Induced by Heterozygous BMP Receptor Deletion in *Drosophila* Wing Development

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Undergraduate student

Organisms randomly accumulate mutations, such as point mutations, insertions, and deletions, throughout life. Most mutations in diploid organisms are recessive, providing no phenotypical effects as a heterozygote. Accordingly, the 'two-hit model' serves as a central conceptual framework in cancer genetics. While the model supports losing two copies of wild-type alleles required for a visible effect, the impacts of heterozygous mutant cells at the tissue level are not fully understood. Our previous findings reveal an unexpected mosaic effect of heterozygous recessive mutant cells. Heterozygous Bone Morphogenetic Protein (BMP) receptor missense mutations exhibit a dominant effect when existing as clones of cells in wing primordial tissues, highlighting the importance of investigating tissue genetics. Here, we examine how a heterozygous deletion mutation of the BMP receptor influences *Drosophila* wing development to expand our knowledge of tissue genetics. We find that introducing heterozygous deletion clones in wing primordial tissue by heat shock-mediated recombination caused more severe wing phenotypes than heterozygous animals by disrupting proper cell-cell communication via BMP signaling. Intriguingly, generating large mutant clones by increasing the duration of the heat shock alleviated the wing phenotypes, probably due to resolving the mosaic condition. These findings indicate that not only missense mutations (changing the protein quality) but also deletion mutations (reducing protein quantity) can cause the heterozygous recessive mosaic effect. Further, while feedback regulation in gene expression provides robustness to the biological system at the organismal level, our results suggest the vulnerability of feedback regulation at the tissue level.

[83B] Influence of genetic variation on obesity in *Drosophila melanogaster* utilizing the AKHR pathway
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Obesity is a serious, chronic disease that is considered a global health issue. There are many factors that contribute to obesity, both environmental and genetic. With the obesity epidemic on the rise, it is important that we develop a better understanding of the genetic impact on this disease. The glucagon signaling pathway is responsible for fat breakdown in humans by stimulating the enzyme lipase, which breaks down fats for energy storage. If this pathway malfunctions, there is a decrease in fat breakdown, resulting in obesity. In *Drosophila melanogaster,* loss of the adipokinetic hormone receptor (*AKHR*), the homologue for the glucagon receptor, induces obesity by leading to less breakdown of stored fats. To determine the genetic influences on AKHR/glucagon signaling, we crossed a model with reduced *AKHR* expression with the Drosophila Genetic Reference Panel (DGRP). Using larval density as a proxy for stored fat, we have identified a number of interpreting candidate modifiers for follow-up studies. Ultimately, our goal is to build on our preliminary results with measurements from all the DGRP strains, utilizing the results to identify therapeutic targets and biomarkers that could be used in humans.

[84B] Genetic Guardians: The Critical Role of Dna2 in Genome Stability
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DNA is essential for the survival and reproduction of living organisms and undergoes constant replication and repair. Mutations, arising from replication errors, mutagen exposure, or health conditions, threaten genomic integrity. The DNA2 gene is crucial for maintaining DNA stability and regulating replication, thus playing a vital role in cellular health. We investigated Dna2 function using Drosophila melanogaster, which shares ~70% genetic similarity with human disease-causing genes. Our goal was to expose Dna2-deficient flies and flies that overexpress Dna2 to various mutagens to understand Dna2's role in DNA repair. Previously, our lab found Dna2-deficient flies exhibit sensitivity to methyl methanesulfonate (MMS), which causes large DNA adducts and impairs replication. We extended this research by investigating how lower doses of MMS influence the sensitivity of Dna2 mutant flies, as well examining the response of Dna2 mutants to the mutagen's bleomycin (double strand breaks), potassium bromate (oxidative stress), and nitrogen mustard (interstrand crosslinks). Our results revealed that different Dna2-deficient alleles showed similar dose-dependent sensitivity to MMS. Dna2 mutants were not sensitive to bleomycin or potassium bromate but were sensitive to nitrogen mustard. To test the hypothesis that lack of Dna2 improves DNA damage tolerance, we treated flies that overexpress Dna2 to MMS, bleomycin, and potassium bromate, and found no difference compared to wild type flies. Together the findings support the role of Dna2 in responding to replication stress but not other types of DNA repair. This research can advance our understanding of DNA repair mechanisms and potentially aid future cancer and disease treatments.

[85A] *Drosophila*'s method of calcium propagation under starvation
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Undergraduate student

Communication between cells is vital for the survival and proliferation of multicellular organisms. In *Drosophila*, starvation triggers the propagation of calcium waves across the fat body allowing communication between fat body cells. Yet, the mechanism of this intercellular signaling is still unclear. Understanding the process behind the starvation response in *Drosophila* is crucial for insight into human health and human starvation processes. This study aims to identify specific proteins involved in calcium waves by using gene knockout. To test the role of gap junctions in calcium wave propagation, clones lacking either *inx1* or *inx2* were generated. The clones were generated using flip-out technology and heat shock *flp* to induce GAL4

expression in around 1:20 cells. GAL4 activated the expression of UAS-Cas9, which in conjunction with the appropriate guide RNAs, caused knockout of *inx1* or *inx2*. The mutant clones were visualized using UAS-CD8-RFP and the calcium waves were visualized using *apoLpp::GCaMP6S*. The larvae were live imaged after 24-hour starvation via a spinning disc confocal. Imaging revealed that *inx1* knockout delayed the entry and exit of calcium in mutant fat body cells. Whereas *inx2* knockout had a greater inhibition; calcium could not enter or exit a mutant cell. These findings suggest that gap junctions play a role in the intercellular communication of starvation in *Drosophila*. Understanding calcium propagation may lead to insights into similar processes in human health, especially in cases where the starvation response is impaired.

[86A] Fate in Focus: Investigating *Dve* and *Chb* Roles in *Drosophila* Eye Development

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Graduate student

Chromosome bows (*Chb*)/*Mast*/*Orbit* is essential for organizing the bipolar mitotic spindle at the kinetochore. This microtubule plus-end tracking protein functions in maintaining the microtubule dynamics, and is evolutionarily conserved, with its human ortholog being cytoplasmic linker-associated protein (CLASP). CLASP is vital for microtubule distribution and stability during the cell cycle and has been implicated in neurofibrillary tangle formation in tauopathies. To mechanistically understand how pathogenic variants in *CLASP/Chb* contribute to developmental delays, we are utilizing *Drosophila* as a genetically tractable model system that encompasses various developmental stages. Our investigation on genetic interactome revealed that *Chb* is suppressed by *defective proventriculus (Dve)*, a K50 homeodomain transcription factor, which is essential for cell type specification and expressed in the dorsal head vertex region of the eye-antennal imaginal disc. We hypothesize that if Dve regulates Chb expression, it may lead to altered microtubule assembly. By employing the UAS-Gal4 system to knock down and overexpress *Chb* in the eye, we expect to observe a reduction in eye size and a shift from eye to head fate in the proper ommatidial arrangement. Additionally, we aim to investigate the effects on *Dve* expression and its downstream regulators of eye development. This research will provide insights into the key partners involved in the cellular assembly of microtubules during eye development.

[87B] The impact of insertion bias into piRNA clusters on the invasion of transposable elements

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Transposable elements (TEs) are traditionally thought to insert randomly in genomes until they encounter piRNA clusters, genomic regions that produce small RNAs that silence TEs. However, some TEs, like the P-element in Drosophila, show preferential insertion into these clusters. This observation raises an interesting question: Could such insertion bias benefit TEs while allowing them to spread through populations? Using forward simulations, we investigated how different levels of TE insertion bias into piRNA clusters affect invasion dynamics and host fitness. Our results show that insertion bias significantly alters TE invasion patterns, primarily by modifying TE copy numbers in individuals before silencing occurs. While insertion into piRNA clusters reduced the harmful effects of TEs on host populations, our simulations revealed that TEs avoiding clusters actually outcompete those with cluster-insertion bias. Interestingly, insertion bias only benefited TEs under specific conditions: when negative selection acted against TEs and recombination was limited. The variation in insertion bias among different TE families suggests this trait is TE-specific rather than host-determined. However, our findings indicate that scenarios where this bias benefits TEs are surprisingly limited. This work challenges our understanding of TE-host dynamics and opens new avenues for investigating insertion bias evolution during TE invasions, particularly in Drosophila where the piRNA pathway plays a crucial role in genome defense.

[88B] Motor Pattern Alterations in a Model of Neurofibromatosis Type 1

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Neurofibromatosis type 1 is a genetic disorder caused by the loss of the neurofibromin protein (Nf1). Neurofibromatosis has a wide range of physical and cognitive manifestations including autism spectrum disorder and attention deficit hyperactivity disorder. Loss of Nf1 function may alter circuit activity, leading to behavior changes. We have found that loss of Nf1 in *Drosophila* alters spontaneous motor behaviors, including increasing grooming frequency. Whether Nf1 deficiency alters the frequency and pattern of sensory-evoked behaviors is unknown. If flies are covered with dust, they engage in vigorous grooming to remove the dust. Here we test the effects of dusting wild-type and *nf1* mutant flies. The data suggest that dusting increases grooming frequency in both genotypes, and that the grooming pattern in *nf1* mutants is altered: the normal sequence/prioritization is scrambled. This suggests that Nf1 loss both increases spontaneous grooming frequency and changes the pattern of this temporally-sequenced motor behavior.

[89A] The Effects of MAST Kinases on Hedgehog Signaling and Compartmentalization

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The *Drosophila melanogaster* wing disc develops into the wing of adult flies after forming distinct compartments. Hedgehog (Hh) signaling facilitates the formation of the anterior/posterior boundary. Using genetic mosaics, we examined the role of Microtubule-Associated Serine/ Threonine (MAST) Kinases on compartmentalization. Fluorescent antibodies allow us to observe the effects of clones homozygous for mutated *drop out* (*dop*), the *Drosophila* MAST Kinase homologue, on Hh pathway proteins and structural proteins within the disc. Clones homozygous for *dop10,* a null mutation, cause changes to Hh pathway protein expression when they are located along the anterior/posterior boundary. Expression of Patched, the Hh protein receptor and a target gene of the Hh pathway, increases as a result of mutant clones on either side of the boundary. This may be due to decreased expression of *engrailed*, a Hh pathway target gene which provides a negative feedback mechanism by acting as a transcription repressor. Additionally, *dop* mutations have been shown to cause defects in microtubule transport which facilitates apical/basal transport within the cell. This leads to disruption of actin projections from the basal surface known as cytonemes, which may play a role in intercellular Hh transport. Staining actin of *dop10* clones revealed that mutant clones appear to send projections to clones on the other side of the boundary and/or are able to cross the anterior/ posterior boundary.