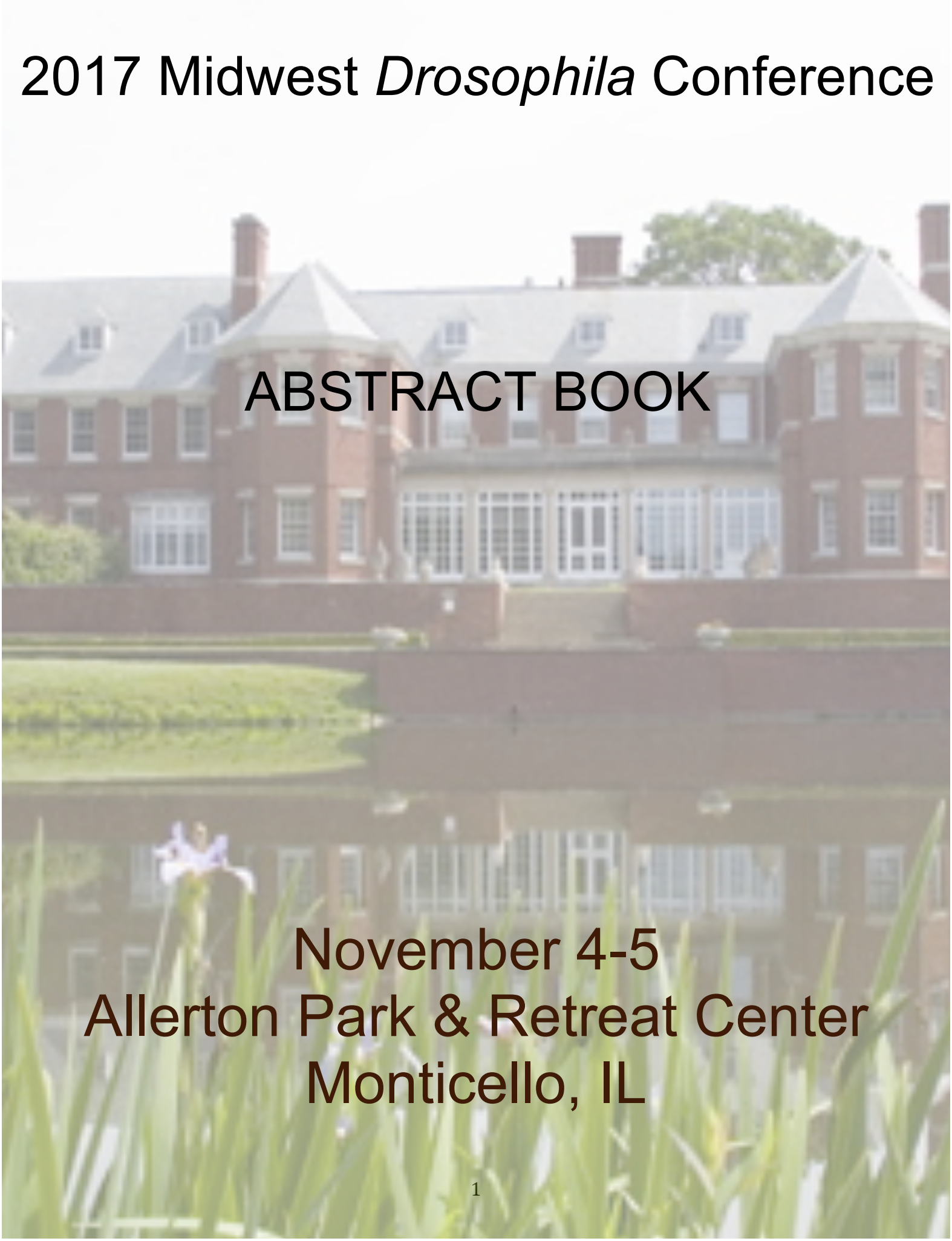
2017 Midwest *Drosophila* Conference



ABSTRACT BOOK

November 4-5
Allerton Park & Retreat Center
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2017 Midwest Drosophila Conference ABSTRACTS

TALKS

[1] **Nrf2 regulates tissue regeneration through ROS and JNK signaling in the *Drosophila* imaginal wing disc**

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Regeneration is a fascinating process that requires an organism not only to recognize and repair tissue damage, but also to grow and pattern replacement tissue. While some regulators of regeneration have been identified, many of the genes and signals required for this process are still unknown. We use *Drosophila* to screen for novel regulators of regeneration using a genetically induced ablation system in the larval wing disc. The power of *Drosophila* genetics makes this system complementary to vertebrate models with robust regenerative responses but less genetic tractability. We conducted a screen of chromosomal deficiencies spanning the right arm of the third chromosome, and identified *cap-n-collar*, the *Drosophila* homolog of vertebrate Nrf2, as an important regeneration gene. Nrf2 responds to the presence of damaging reactive oxygen species (ROS) in many contexts by promoting transcription of antioxidant enzymes to quench ROS levels. However, ROS are an important signal after tissue damage and an activator of the JNK signaling pathway, a well-known regulator of tissue repair. We determined that ROS levels were higher in Nrf2 heterozygotes during regeneration, suggesting that while moderate levels of ROS are required for regeneration, high ROS levels are detrimental to tissue repair. Nrf2 mutants also displayed defects in wound closure, initial proliferation, and coordination of regeneration and development, which are all regulated by JNK signaling. Interestingly, JNK signaling was reduced in damaged Nrf2 heterozygotes, suggesting that JNK activation requires a specific range of ROS levels. To determine the dose-dependent relationship between ROS and JNK activity, we exposed regenerating wild-type wing discs to ectopic ROS and found that high ROS levels alone were able to dampen the JNK signal. Here we present a model wherein Nrf2 is required to restrain ROS signaling to maintain the optimal levels for JNK activation and tissue regeneration.

[2] **Metabolomic analysis reveals that the *Drosophila* gene *lysine* influences diverse aspects of metabolism**

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The fruit fly *Drosophila melanogaster* has emerged as a powerful model for investigating the molecular mechanisms that regulate animal metabolism. A major limitation of these studies, however, is that many metabolic assays are tedious, dedicated to analyzing a single molecule, and rely on indirect measurements. As a result, *Drosophila* geneticists commonly use candidate gene approaches, which, while important, bias studies towards known metabolic regulators. In an effort to expand the scope of *Drosophila* metabolic studies, we used the classic mutant *lysine* (*lys*) to demonstrate how a modern metabolomics approach can be used to conduct forward genetic studies. Using an inexpensive and well-established gas chromatography-mass spectrometry (GC-MS)-based method, we genetically mapped and molecularly characterized *lys* by using free lysine levels as a phenotypic readout. Our efforts revealed that *lys* encodes the *Drosophila* homolog of Lysine Ketoglutarate Reductase/Saccharopine Dehydrogenase (LKRSDH), which is required for the enzymatic degradation of lysine. Furthermore, this approach also allowed us to simultaneously survey a large swath of intermediate metabolism, thus demonstrating that *Drosophila* lysine catabolism is complex and capable of influencing seemingly unrelated metabolic pathways. Overall, our study highlights how a combination of *Drosophila* forward genetics and metabolomics can be used for unbiased studies of animal metabolism and demonstrates that a single enzymatic step is intricately connected to diverse aspects of metabolism.

[3] **Tumor suppressive roles of Nucleoporins 98 and 96 in Drosophila wing epithelium**
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The Nucleoporin 98KD (Nup98) is one of the most promiscuous translocation partners in hematological malignancies, contributing to at least 31 different truncation-fusion proteins. To date, nearly all disease models of Nup98 translocations involve ectopic expression of transgenes recapitulating the fusion protein under study, leaving the endogenous Nup98 loci unperturbed. Hence, they cause either the upregulation or the downregulation of Nup98 activity. The contribution of Nup98/96 to the leukemic disease phenotype remains unknown. Nup98 and 96 are also mutated in many other cancer types and located near a tumor suppressor region known to be epigenetically silenced, suggesting that their disruption may not be limited to blood cancers. We found that reducing Nup98/96 function via an RNAi approach in *Drosophila melanogaster* (where the Nup98/96 shared mRNA and reading frame gene structure is conserved) de-regulates the cell cycle. We find evidence of overproliferation in Nup98/96-deficient tissues, counteracted by elevated apoptosis and aberrant Wingless, TNFa and JNK signaling associated with wound healing. When the knockdown of Nup98/96 is combined with inhibition of apoptosis, we see synergism leading to overgrowth consistent with a tumor-suppressor function for endogenous Nup98 and/or 96. We have found that the loss of Nup98/96 activates JNK stress signaling due to defects in the nuclear export of a ribosomal protein RPL10. This leads to a paradoxical state of decreased protein synthesis yet increased proliferation in Nup98/96 knockdown cells. Importantly, the overexpression of Nup98/96 also leads to defects in RPL10 localization, JNK signaling and aberrant proliferation. Based upon our data, we suggest that Nup98/96 act as a 'Goldilocks' gene in blood cancers, where too much or too little leads to tumorigenic overproliferation that can cooperate with other mutations in cancer.

[4] **Investigating the roles of Fascin in collective cell migration using Drosophila border cell migration**
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Fascin, an actin binding protein, is a regulator of many developmental processes and contributes to cancer aggressiveness. Functioning to bundle actin filaments, Fascin promotes cell motility, invasion, and adhesion through its canonical role of forming filopodia and invadopodia. Fascin controls cell migration during development such as, growth cone extension and dendrite formation. In addition, Fascin is highly upregulated in certain types of cancer, and elevated expression is associated with increased invasiveness, aggressiveness and mortality of these cancers. While Fascin's role in regulating cell migration has largely been attributed to its function as an actin bundler, Fascin has other functions including, interaction with mechanotransduction machinery, and nuclear localization, that may contribute to cell migration. While Fascin has been studied in the context of single cell migration and 2D migration, the role of Fascin in 3D collective cell migration has yet to be investigated. To study the role of Fascin in invasive, collective cellular migration *in vivo* we use *Drosophila* border cells as a model. Border cell migration occurs during Stage 9 of oogenesis in which a specified group of follicular epithelial cells cluster together and migrate posteriorly in between the nurse cells to the nurse cell - oocyte border. This process is crucial for oocyte development since aberrant or delayed border cell migration leads to female sterility. Fascin is highly expressed in the border cells and *fascin*-null flies are sterile. These and other findings led us to hypothesize that Fascin plays a critical role in promoting border cell migration during oogenesis. Contrary to prior reports, we find that follicles from young *fascin*-null flies display a significant delay in border cell migration. Cell-specific knockdown studies suggest that Fascin is required within the somatic cells or specifically the border cells to mediate migration. Furthermore, rescue of Fascin expression in the germline cells, but not somatic cells, fails to rescue border cell migration. Together these findings implicate a cell-autonomous role for Fascin in collective cell migration *in vivo* during *Drosophila* oogenesis. Furthermore, these findings provide a

system to investigate the actin bundling-independent functions of Fascin in invasive cellular migration. Overall, this research will lead to a more complete understanding of the function of Fascin in developmental cell migrations and cancer metastasis.

[5] **Protein Phosphatase 1 is a switch for single cell to collective cell migration**

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Collective cell migration is critically important in embryo development, wound healing, adult tissue renewal and cancer metastasis. While extensive research has been conducted in a variety of models, the underlying mechanisms of collective cell migration are still poorly understood. *Drosophila* border cells undergo a developmentally regulated collective cell migration during oogenesis. Border cells travel as a cohesive cluster of 6-10 cells between large “nurse cells” to reach the oocyte. While roles for several serine-threonine kinases and their target effectors have been established in border cell migration, much less is known about serine-threonine phosphatases. Here we show that Protein Phosphatase 1 (PP1) maintains the collective cohesion and migration of border cells. Inhibition of PP1 activity, either through the endogenous inhibitor NiPP1, or by knockdown of multiple PP1 catalytic subunits, causes border cells to round up and completely dissociate from the cluster during their migration, which can be rescued by overexpressing PP1 catalytic subunits. These individual border cells also have slower overall movement, with protrusions that form randomly between cells. Rac activity is still enriched in the leading border cell, showing that overall guidance signaling and directionality are not affected. However, levels of E- cadherin between cells are strongly reduced. Non-muscle myosin II (myo-II) localization is also altered and F-actin is enriched around individual border cells rather than at the periphery of the entire border cell cluster. Consistent with previous work from the lab on the role of myosin phosphatase, phosphorylated myo-II regulatory light chain is increased in individual border cells. Together, these cellular alterations contribute to the failure of PP1-inhibited border cells to move as a group. Thus, PP1 activity promotes a collective rather than individual mode of cell migration.

[6] **The role of prostaglandins in collective, invasive cell migration**

Emily Fox and Tina Tootle

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Collective cell migration – the coordinated movement of tightly or loosely associated cells – is important for both normal development and tumor invasion. While prostaglandins (PGs), short-range lipid signaling molecules, regulate cell migration, their mechanisms of action are poorly understood in both single and multicellular migration contexts. To address this knowledge gap we use the collective, invasive, epithelial migration that occurs during *Drosophila* oogenesis. The *Drosophila* ovary contains chains of developing follicles composed of 15 germline derived nurse cells and 1 oocyte surrounded by a layer of somatic epithelial cells. During Stage 9 of oogenesis, a cluster of 6-8 of these somatic cells delaminate from the outer epithelium and migrate invasively between the nurse cells to the oocyte border; this migration is termed border cell migration. To study the roles of PGs in border cell migration, we utilize genetic mutations in *pxt*, the Drosophila cyclooxygenase-like enzyme, which is responsible for all PG synthesis. Using quantitative analyses, I find that loss of Pxt causes aberrant border cell migration. Loss of Pxt results in both a significant delay in border cell migration and an increase in cluster length compared to wild-type controls. We hypothesize that both the delay and alteration in cluster morphology are due to changes in among the border cells and/or between the border cells and the surrounding nurse cells. While E-Cadherin appears to be unaffected by the loss of Pxt, integrin levels on the interface between the border cells and the nurse cells is reduced. As integrin-based adhesion is essential for correctly timed border cell migration and cluster co-hesion, our data supports that model that PGs regulate integrins to control border cell migration and cluster morphology. Future

work will further investigate this model as well as the role of PGs in the border cell cluster vs the nurse cells. Our studies on PG signaling during border cell migration provide insights into the conserved mechanisms by which PGs regulate collective, invasive cell migrations. Indeed, high levels of PGs and integrins are independently associated with cancer migration and metastasis.

[7] **Comparative Meiotic Cytology Among Drosophila Species**
Ahmed Majekodunmi and William Gilliland
DePaul University Department of Biological Sciences

The Muller F element can be found in many fruit fly species as a very small 'dot' chromosome, although some species (e.g. *D. willistoni)* have lost this chromosome through fusion with another chromosome. The exact reason why flies have this small chromosome is not known, however the two dot homologs move out onto opposite sides of the meiotic spindle during female meiotic prometaphase I, being positioned between the spindle poles and the chromosomes at the metaphase plate. A previous manuscript from our lab identified a difference in the dot chromosome positioning between *D. melanogaster* and *D. simulans*, where the dots in the former species move out around twice as far onto the spindle on average. It was speculated that the difference could correlate with the amount of heterochromatin in the genome (*D. melanogaster* has around 25% more than *D. simulans*) or with the abundance of inversions (*D. melanogaster* is polymorphic, while *D. simulans* is monomorphic). To test this idea, we have measured dot positioning in 12 additional species (6 monomorphic and 6 polymorphic for inversions). We found no significant correlation between either heterochromatin content or inversion type and the dot-dot distances among these species. However, we did discover over 10-fold differences in the apparent sizes of the dot chromosomes, and over 5-fold differences in the rates of chromosomes being out on the spindle, and these two factors are strongly correlated ($r = 0.79$). Because this data is from fixed images, we interpret these results to mean that the size of the dot chromosome is highly correlated with the length of time spent doing these meiotic prometaphase chromosome movements.

[8] **CRISPR/Cas9-mediated mutagenesis reveals a novel functional domain of Zelda essential for development**
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In nearly all metazoans, the earliest stages of development are controlled by maternally deposited mRNAs and proteins. The zygotic genome becomes transcriptionally active hours after fertilization. Transcriptional activation during this maternal-to-zygotic transition (MZT) is tightly coordinated with the degradation of maternally provided mRNAs. In *Drosophila melanogaster,* the transcription factor Zelda plays an essential role in widespread activation of the zygotic genome. While Zelda expression is required both maternally and zygotically, the mechanisms by which it functions to remodel the embryonic genome and prepare the embryo for development remain unclear. Using Cas9-mediated genome editing to generate targeted mutations in the endogenous *zelda* locus, we determined the functional relevance of protein domains conserved amongst Zelda orthologs. We showed that neither a conserved N-terminal zinc finger nor an acidic patch were required for activity. Similarly, a previously identified splice isoform of *zelda* is dispensable for viability. By contrast, we identified a highly conserved zinc- finger domain that is essential for the maternal, but not zygotic functions of Zelda. Animals homozygous for mutations in this domain survived to adulthood, but embryos inheriting these loss-off-function alleles from their mothers died late in embryogenesis. These mutations did not interfere with the capacity of Zelda to activate transcription. Unexpectedly, these mutations generated a hyperactive form of the protein and enhanced Zelda-dependent gene expression. These data have defined a protein domain critical for controlling Zelda activity during the MZT, but dispensable for its roles later in development, for the first time separating the maternal and zygotic requirements for Zelda. This demonstrates that highly regulated levels of Zelda activity are exclusively required for establishing

the developmental program during the MZT. We propose that tightly regulated gene expression is essential to navigate the MZT and that failure to precisely execute this developmental program leads to embryonic lethality.

[9] **Update from the DGRC**
Andrew Zelhof, Director
Indiana University

[10] **Update from the BDSC**
Cale Whitworth, Collections Manager and Scientific Staff
Indiana University

[11] **Circadian Regulation of Drosophila Feeding Behavior**
Austin P. Dreyer, Madison Martin, Daniel Jabr, Daniel Cavanaugh
Loyola University of Chicago

Biological organisms are intimately connected to the 24-hour cycling of environmental conditions by endogenous molecular clocks. These internal clocks are responsible for coordinating a range of physiological and behavioral responses with environmental cycles to ensure proper organismal function. How circadian responses are regulated as outputs is an area of active research, particularly in light of the negative health effects associated with chronic deviation from normal circadian rhythms. Here, we present one of the first dissections of how feeding rhythms, key contributors to the maintenance of metabolic health, are regulated in *Drosophila*. A primary goal of our research is determining how circadian information generated in central brain clock cells by the molecular clock is propagated to output cells to control feeding behavior. One population of cells directly related to metabolism and shown to interface with the central clock are the insulin producing cells (IPCs) that express *Drosophila* insulin-like peptides (DILPs) 2, 3, and 5 in adult flies. It was recently demonstrated that these cells exhibit clock-dependent oscillations in firing rate, and we therefore hypothesized that this may enable them to modulate feeding behavior in a circadian manner. To test their contribution to circadian feeding rhythms, we used the newly-developed Fly Liquid-Food Interaction Counter (FLIC) system to monitor feeding in real time over the course of several days in flies in which we induced adult-specific constitutive activation or inactivation of Dilp2-expressing neurons. These manipulations should eliminate circadian firing patterns, however, we found no effect on feeding rhythm strength, arguing against a role for these cells in regulating feeding rhythms. In addition to central clock cells in the brain, molecular clocks are present in peripheral tissues and have been shown to influence circadian responses in tissue-specific functions. To dissect the relative contribution of central and peripheral clocks to feeding rhythms, we altered the speed of molecular clocks in central clock neurons of the brain and in the fat body, a peripheral clock tissue with metabolic function. We found only changes in the speed of central clock cells in the brain had an effect on feeding rhythms, suggesting circadian feeding is a tightly regulated behavior with possible redundancies in control mechanisms.

[12] **Correlating lifespan with sleep architecture in Drosophila**
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The link between health and good sleep is well known - but there is still much to learn about the biological mechanisms that govern the sleeping process and how changes in this system affects an organism's overall health. Previous efforts that examine basic sleep metrics have resulted in limited success either describing or predicting lifespan in *Drosophila melanogaster*. . But since there are increasing reports linking sleep with health, we hypothesize that sleep architecture is unique to each individual animal and that changes in these characteristics over time may be more indicative of the animal's health than basic sleep metrics alone. For example, longer sleep is not necessarily better, and

it's likely that the duration of sleep needed is unique to each individual animal, with variation even within the same species.

We have been collaborating with investigators in the Department of Statistics to develop a sophisticated model that generates lifespan predictions based on fly wake and sleep transition data, along with changes in general sleep stability. We utilized *Drosophila* Activity Monitors (DAM System; Trikinetics) to track individual fly movement patterns, and built a model using sleep data from over 400 flies. Using this model, we subsequently binned flies into short-lived and long-lived cohorts after a 30 day monitoring period, allowing us to determine how biomolecular processes change with an organism's age. Using these methods, we have identified a difference in oxidative stress makers between our long- and short-lived flies.

[13] **An Investigation into the Behavioral and Physiological Effects of Chronic Circadian Misalignment in Drosophila melanogaster**

Alex Boomgarden, Gabriel Sagewalker, Pramathini Patel and Daniel Cavanaugh
Loyola University Chicago

As a result of earth's orientation toward the sun producing day and night, organisms have evolved an endogenous circadian timing system that is responsible for the 24-hour oscillation of most physiological and behavioral processes. This timing system is constantly synchronized to the external environment to adapt to and anticipate changes in light, temperature, food, and mate availability. In modern society, social and work constraints cause people to live schedules that are out of sync with their internal circadian clocks, producing a chronic circadian misalignment. While epidemiological studies in humans point to potentially damaging metabolic and cognitive consequences of chronic circadian misalignment, the full extent of these negative effects is unknown. Furthermore, very little is known about the molecular and cellular mechanisms that lead to the negative effects of circadian disruption. Here, we model and investigate the consequences of chronic circadian misalignment by exposing the flies to a 28-hour day comprised of 14 hours of light and 14 hours of dark (compared to control flies that are exposed to a standard 24-hour day). Using the Drosophila Activity Monitoring (DAM) system, we can subject flies to such schedules while continuously monitoring their locomotor activity. This allows us to assess the effect of long-term circadian misalignment on aging-associated changes in average locomotor activity levels, circadian rhythm strength, and sleep, and to correlate these measures with fly lifespan. We demonstrate that exposure of flies to the 28-hour schedule led to an 11.5% reduction in lifespan in the females and a 16.3% reduction in males. While misaligned flies exhibited aberrant timing of locomotor activity, evidenced by reduced rest:activity rhythm strength, we found no differences in overall locomotor activity or sleep, indicating that the reduction in lifespan was independent of these behaviors. Currently, we are assessing the effect of chronic circadian misalignment on the cellular stress response of the fly by monitoring expression of stress-related genes through the use of reporter lines. We believe that these studies will provide insight into the mechanisms underlying the reduction in longevity caused by circadian misalignment.

[14] **Serotonin signaling ties psychological experience with healthy aging in Drosophila**

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Sensory perception modulates health and aging across taxa. Understanding the nature of these cues and the mechanisms underlying their action may lead to novel interventions that improve the length and quality of life. In humans, psychological trauma is often associated with the recognition of dead individuals, with chronic exposure leading to persistent mental health issues including depression and post-traumatic stress disorder. The mechanisms that link mental and physical health, and the degree to which these are shared across species, remain largely unknown. Here we show that the vinegar fly, *Drosophila melanogaster*, has the capability to perceive dead conspecifics in its environment and that

his perceptive experience induces both short- and long-term effects on health and longevity. Death perception is mediated by visual cues, and remarkably, its effects on healthy aging are eliminated by targeted attenuation of serotonin signaling. Our results establish a complex perceptive ability in *Drosophila* that reveals deeply conserved mechanistic links between psychological state and aging, the roots of which might be unearthed using simple model systems.

[15] **The Drosophila SK Potassium Channel Negatively Regulates Nociception**
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Inhibition of nociceptor activity is important for the prevention of spontaneous pain and hyperalgesia. To identify the critical K^+ channels that regulate nociceptor excitability we performed a forward genetic screen using a *Drosophila* larval nociception paradigm. Knockdown of three K^+ channel loci, the *small conductance calcium-activated potassium channel (SK), seizure,* and *tiwaz,* resulted in marked hypersensitive nociception behaviors. In more detailed studies of *SK*, we found that hypersensitive phenotypes could be recapitulated with a genetically null allele. Importantly, the null mutant phenotype could be rescued with tissue specific expression of an *SK* cDNA in nociceptors. *SK* showed expression in peripheral neurons and interestingly SK proteins localized to axons of these neurons and were not detected in dendrites. Optical recordings from nociceptive neurons showed a significant increase in mechanically activated Ca^{2+} signals in SK mutant nociceptors. Our findings suggest a major role for SK channels in the regulation of nociceptor excitation.

[16] **Drosophila tafazzin mutants have reduced exercise capacity**
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Cardiolipin (CL) is a mitochondrial phospholipid that helps maintain the curvature of the mitochondrial membrane and stabilize the protein complexes of the electron transport chain to promote efficient ATP synthesis. Tafazzin, an acyl-transferase, is required for synthesis of the mature form of CL. Mutations in the *tafazzin* (Taz) gene are associated with a human disorder known as Barth syndrome (BTHS). Symptoms of BTHS often include muscle weakness and exercise intolerance. Previous work demonstrates *Drosophila* Taz mutants exhibit motor weakness resulting in reduced flying and climbing abilities. However, Drosophila Taz mutants' response to exercise has not been examined. In this study, we examined the baseline exercise capacity of Taz mutant flies, and their ability to adapt to exercise training. Prior to training, Taz mutants demonstrated reduced endurance and flight capabilities relative to control flies. After training, exercised Taz mutants' endurance and flight ability improved, though their performance was not fully rescued to wild-type levels. Although cardiac phenotypes are observed in human patients, no obvious cardiac phenotype was observed in *Drosophila* Taz mutants. In the future, we hope to use endurance as a novel screening tool to identify genetic modifiers of Taz.

[17] **The protective effect of mitochondria complex I knockdown in a Drosophila model of chemotherapy-induced peripheral neuropathy**
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Chemotherapy-induced peripheral neuropathy (CIPN) is a common, severe, dose limiting side effect of platinum-based chemotherapeutics such as cisplatin, affecting approximately 30% of those treated. Currently, there are no effective treatments or preventive measures for this lifelong side effect. Additionally, there are no reliable predictive measures to identify those patients most likely to develop CIPN. As such, there is a strong need to better understand the mechanisms leading to peripheral nerve damage caused by cisplatin and to understand the factors contributing to CIPN susceptibility. *Drosophila melanogaster* provides an excellent model system with which to address these questions.

We have developed a model of CIPN using adult *Drosophila*. When flies are fed cisplatin in a 10% sucrose solution, cellular damage and apoptosis - observable via immunofluorescence - occur in the two primary cell types that are damaged in human patients: neurons and rapidly dividing cells. Damage to neurons can be assessed using a negative geotactic climbing assay, making the system amenable to semi-high throughput screening. Female ovaries (containing rapidly dividing cells) are also strongly damaged by cisplatin, providing an internal positive control for cisplatin activity. Using this system, we developed an RNAi-based screen to identify novel genes that affect *Drosophila* sensitivity to CIPN. Here, we demonstrate that RNAi-mediated knockdown of genes in neurons can alter *Drosophila* sensitivity to cisplatin. Genes that have been previously associated with CIPN sensitivity in humans, including glutathione peroxidase and glutathione synthetase, also affect cisplatin sensitivity in flies, showing that our approach can identify conserved genes. Additionally, we have identified a novel gene that affects *Drosophila* sensitivity to cisplatin. We show that genetic reduction of ND13A, a mitochondria complex I subunit, protects flies from cisplatin-induced neuronal damage. Protection against cisplatin is not specific to the ND13A subunit, as multiple complex I subunits result in similar protection when knocked down. However, knockdown of subunits from complexes II, III, and IV is not protective against cisplatin. These data demonstrate the potential of mitochondria complex I inhibition as a therapeutic target to prevent CIPN.

[18] **Mechanical stress dissipation in the Drosophila wing imaginal disc through calcium signaling**

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Organ development depends on the tight coupling between biochemical and mechanical cues. Calcium (Ca^{2+}) ions are key integrators of chemical and mechanical inputs. However, Ca^{2+} signaling is poorly understood in developing epithelia. Further, very few tools exist to study mechanotransduction at millimeter length scales. Here, we report advances in organ-level experimentation. We used advanced genetic tools available in a fruit fly-based model of organ growth, the larval wing imaginal disc, to study the regulation and function of intercellular Ca^{2+} waves (ICWs), which occur during organ growth. We developed a fluidic device called the Regulated Epithelial Microenvironment Chip. This microfluidic device enables modulation of multiple stimuli acting on micro-organs. The system also enables live imaging of cell signaling responses. This approach enabled us to decouple the contributions of biochemical signaling and mechanical loading to Ca^{2+} signaling dynamics. We discovered that stimulation of ICWs depend upon the pre-loading signaling activity, rather than the magnitude or duration of mechanical loading. ICWs rely on calcium induced calcium release and propagation through gap junctions. ICWs are also stimulated with a loss of extracellular matrix and subsequent relaxation of tissue stresses. In vivo and ex vivo observations demonstrate that the Ca^{2+} signaling dynamics correlate with organ growth rates and are downstream of morphogenetic signaling. Perturbations to Ca^{2+} signaling lead to distortions in tissue folding. This process depends on non-muscle Myosin II. Deregulation of Ca^{2+} signaling also impacts the final wing architecture. These efforts provide quantitative insights into how Ca^{2+} signaling integrates multiple stimuli to module cellular properties such as actomyosin contractility and cell adhesion. Together, these results provide support for a “Mechanical Stress Dissipation” hypothesis of Ca^{2+} signaling. This hypothesis states that mechanical tension release during organ growth generates intercellular Ca^{2+} transients to modulate the shape of the final organ architecture through non-cell autonomous regulation of actomyosin contractility. These studies help identify strategies for spatiotemporally programing Ca^{2+} signaling during organ growth. Such advances in calcium signaling engineering can lead to novel approaches to target cancer, accelerate regeneration, or advance organoid culture technologies.

[19] **Knockdown of multicopper oxidase 4 Eliminates the Peritrophic Matrix and Alters the Adult Microbiome in Drosophila**

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Extracellular epithelial barriers allow organisms to regulate their interactions with the environment; one dynamic site of such interactions is the gut. Like many insects, *Drosophila melanogaster* produces an extracellular structure called the peritrophic matrix (PM), which lines the midgut and is theorized to aid in digestion, microbiome maintenance and organismal defense. However, an absence of fly lines that lack a PM limits investigation of its physiological role. An RNAi screen of genes encoding putative cross-linking enzymes now reveals that silencing *multicopper oxidase 4* (*mco4*) in the cardia, the site of PM synthesis, produces flies that lack a PM. *mco4* encodes a laccase and is expressed at high levels and nearly exclusively in the cardia, providing a novel and ideal model to study PM function. In adults, knockdown of *mco4* reduced microbiome abundance by 850-fold, as measured by 16S qPCR, and augmented the loss of gut bacteria caused by daily transfer of flies to fresh media. Additionally, the microbial composition of *mco4* knockdowns lacked a significant amount of acetobacter, normally the dominant component of the microbiome. At the same time, expression of the antimicrobial peptide *diptericin* and the cytokine *upd3* were increased 10-20 fold in knockdown flies, indicating hyperactivation of the immune system and response to tissue damage. Raising *mco4* knockdown flies in axenic conditions nearly eliminated the activation of the immune system, suggesting the absence of the PM does not directly induce activation of the immune system in normal conditions. Knocking down *mco4* on an *IMD* mutant background resulted a bacterial abundance that was higher than immune-intact *mco4* knockdowns but lower than *IMD* mutant controls, suggesting a role for the PM both in protecting commensal bacteria from the immune system and in supporting microbiome abundance independent of the immune system. These results support a critical role for the PM in the maintenance of the adult gut microbiome and overall gut homeostasis. Supported by NSF grant IOS-1355087.

[20] **The Evolution of p38K MAP Kinases**

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The highly conserved MAP Kinases, ERK, JNK, and p38K, play an important role in a variety of cellular processes from differentiation to apoptosis. In Drosophila melanogaster, there are three p38K genes: p38Ka, p38Kb, and p38Kc. Upon sequence analysis, we find p38Ka and p38Kb are 78% identical and have an overall similarity of 92%, while p38Kc has accumulated a number of differences that affect critical residues in the TGY motif and kinase domain that are necessary for canonical p38K function. In addition, the p38K genes have distinct roles in the fly but there is also some redundancy between p38Ka and p38Kb. In order to better understand these differences, we have analyzed the evolution of the p38K genes across the sequenced fly species. The p38Ka and p38Kb genes are found across all *Drosophila* species, however p38Kc appears to have arisen during the split between the Willistoni and Obscura groups. Interestingly, *D. psuedoobscura* has unique features with a truncated p38Kc gene and a second p38Kb gene. Looking at a ratio of nonysonymous changes to synonymous changes, we find that all three p38K genes are under purifying selection, though p38Kc is under weaker purifying selection than both p38Ka and b. To further explore the differences between p38Ka and p38Kb, we compared the 1kb upstream region of each gene across multiple Drosophila species. This comparison identified three transcription factor binding sites for the p38Ka gene which were found to be highly conserved across species. Two of these binding sites are of different isoforms of Lola and the third is a homeobox binding site. For p38Kb, we identified a predicted AP-1 binding site and binding sites for two different Lola isoforms as well. Since we have previously shown that p38Kb is a regulator of the oxidative stress response in flies, we were interested in how Lola and AP-1 might be regulating p38Kb as Lola has also been previously linked to oxidative stress. Upon qPCR analysis, we have determined that AP-1 regulates p38Kb expression under normal conditions and lola PT acts under oxidative stress

conditions. Currently, we are performing survival assays to determine how well the Lola and AP-1 flies survive under oxidative stress conditions.

[21] **Identification of a Novel Regulator of Glial Development**
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Glial cells perform numerous functions to support neuron development and function, including axon wrapping, formation of the blood brain barrier, and enhancement of synaptic transmission. We have identified a novel gene, *raw*, which functions in glia of the central and peripheral nervous systems. Reducing *raw* levels in glia results in morphological defects in the brain and ventral nerve cord, and crawling assays reveal that larvae exhibit reduced locomotion, demonstrating defects in neuron function. Examination of the number of glia along peripheral nerves reveals a reduction in glial number upon *raw* knockdown. The reduced number of glia along peripheral nerves occurs as a result of decreased glial proliferation and increased cell death. As Raw has been shown to negatively regulate JNK signaling in other developmental concepts, we examined the expression of the downstream Jun target, *matrix metalloprotease 1* (*mmp1*), and find that *raw* knockdown results in an increase in *mmp1* levels. These results are consistent with previous studies showing increased Mmp levels lead to nerve cord defects similar to those observed upon *raw* knockdown. In addition, knockdown of *puckered,* a negative feedback regulator of JNK signaling, also causes a decrease in glial number. Thus, our studies have resulted in the identification of a new regulator of glial development, and demonstrate that increased JNK signaling negatively impact glial development.

[22] **Understanding the role of Wg Signaling pathway in Aβ42 mediated neurodegeneration**
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Alzheimer's disease (AD), a common form of dementia and an age related progressive neurodegenerative disorder, manifests as memory loss and reduced cognitive ability. One of the hallmarks of AD is formation of the Amyloid-beta 42 (hereafter Aβ42) plaques, which triggers oxidative stress due to aberrant signaling and finally results in the death of neurons. However, the exact mechanism causing cell death is still not well understood. We misexpressed high levels of human Aβ42 protein in the developing fly retina, which mimics AD like neuropathology. In a forward genetic screen, we identified members of highly conserved Wingless (Wg) signaling pathway as modifiers of the Aβ42 mediated neurodegeneration. Misexpression of negative regulator of Wg like Shaggy kinase (sgg) or a dominant negative form of Drosophila T-cell factor ($dTCF^{DN5}$) or blocking Wg transport specifically by downregulating Porcupine (using $porcupine^{RNAi}$) rescued Aβ42 mediated neurodegeneration by reducing the number of dying cells and restoring the axonal targeting from the retina to the brain. It is also known that Wg induces cell death in the early eye developmental stage of *Drosophila.* We therefore want to understand by what mechanism and in which cells the Wg signaling is triggering cell death, whether it's the Aβ42 misexpressing cells or the neighboring wild type cells. In order to approach this question we have developed a two clone system in our lab to understand the crosstalk between the two cell populations, where we have shown that the wild type neighboring cells are undergoing cell death compared to the Aβ42 misexpressed cells.

[23] **Anesthetics influence mortality in a Drosophila blunt trauma model**
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Exposure to anesthetics is common in the majority of early survivors of life-threatening injury. Whether and to what degree general anesthetics (GAs) influence outcome from major trauma is unknown. Because of the requirement for humane treatment of laboratory animals, trauma models in vertebrates almost invariably incorporate anesthesia during the infliction of trauma. Potential confounding effects of GAs (drugs with numerous molecular targets) on outcome measures are commonly disregarded.

We used a blunt trauma model with associated traumatic brain injury (Katzenberger *et al. PNAS* 2013; *JoVE* 2015; *Elife* 2015; *G3* 2016) in *Drosophila melanogaster* to test the hypothesis that general anesthetics modulate outcome from blunt trauma. We administered a standard dose of anesthetics (concentration x duration) either before, during or after a high-impact acceleration-deceleration injury that was calibrated to result in a 24-hour mortality of 20-25%. We found that isoflurane reduced 24-hour mortality when administered before trauma while sevoflurane had a similar but lesser effect. Administration of isoflurane but not sevoflurane after trauma increased mortality at 24 hours.
We conclude that general anesthetics are not neutral with respect to outcome after life-threatening injury and their use should be considered in the interpretation of results obtained in vertebrate trauma models.

POSTERS

1. A transcriptional mechanism controls expression of temporal patterning factor Sloppy-paired in *Drosophila* medullary neuroblasts

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Temporal patterning of progenitor cells –whereby progenitors express a distinct set of transcription factors as they age and each transcription factor specifies daughter cells with distinct fates- is an important mechanism for generating neuron diversity in metazoans. However, the molecular events underlying the sequential expression of patterning transcription factors in defined temporal sequences are insufficiently known. In the present study we have attempted to understand the mechanistic basis of one such temporal transition in neural progenitor cells (neuroblasts) of *Drosophila melanogaster*. Previous work has demonstrated that neuroblasts in the optic lobe of *D. melanogaster* are temporally patterned by transcription factors Homothorax (Hth), Eyeless (Ey), Sloppy-paired (Slp), Dichaete (D) and Tailless (Tll) expressed in the order stated. The present work furthers our knowledge of how an early transition in this cascade- that from Ey to Slp expression is facilitated. Using enhancer-Gal4>>UAS-GFP screens and GFP reporter assays we have identified a 257 bp long sequence in the upstream un-transcribed region of the *slp* locus that drives GFP expression in the same pattern as endogenous *slp* gene in the larval medulla. Further examination of this sequence revealed conserved binding sites for transcription factors Ey, Slp and the Notch (N) co-activator Suppressor of hairless (Su(H)) that were then validated by mutagenesis of the 257 bp sequence. The binding site of Su(H) partially overlaps with that of Slp. It is known that while N is cleaved and N intracellular domain can activate transcription together with Su(H) in neuroblasts, it is membrane tethered and therefore unavailable for transcription in the intermediate progenitors called ganglion mother cells (GMCs). Our findings indicate that Ey, N signaling and Slp itself together control expression of Slp in medulla neuroblasts at the level of transcription. We hypothesize that while Ey is required for initiating expression of Slp in medulla neuroblasts, up-regulation of Slp expression in older neuroblasts requires the co-operation of Ey, N and Su(H). After a critical concentration of Slp has been attained, Slp may displace Su(H) from its binding site on the 257 bp *slp* enhancer and regulate its own expression via a feed-forward mechanism. According to our proposed model, Su(H) should repress *slp* transcription in absence of N signaling such as in GMCs until high level of Slp displaces Su(H) from its own enhancer. The co-expression of Ey and Slp in a subset of medulla neuroblasts is in sharp contrast to their complete segregation in post-mitotic neurons born from these neuroblasts. Importantly, if true, our model would adequately explain this difference in expression patterns as a consequence of differential N signaling in neuroblasts and in the GMCs that are the immediate precursors of neurons. In future we will test predictions of the model that presence of N and its cooperation with Su(H) are critical for transcription of *slp* mRNA and that Su(H) and Slp compete for binding to the 257 bp *slp* enhancer, using genetic and biochemical experiments.

2. The role of p53 isoforms in the *Drosophila* female germline

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We are using Drosophila as a simplified genetic system to define the functions of different p53 isoforms. Vertebrates encode three p53 paralogs – p53, p63, and p73, and each encode numerous protein isoforms that can form many types of heterotetramers composed of subunits from the same or different paralogs. Emerging evidence suggests that these different types of heterotetramers represent a “p53 code” with different biological functions, but the complexity of subunit combinations has stymied interpretation. The *Drosophila* genome has only one p53 ortholog, but, like its vertebrate orthologs, it encodes at least three protein isoforms. Using p53 isoform-specific mutants, we previously showed that the p53A protein isoform is necessary and sufficient for the apoptotic response to radiation. Although a

larger p53B isoform was not required for radiation response, it contains a larger N terminal transactivation domain, and was a more potent inducer of transcription and cell death when over-expressed. This leaves open the question – what is the normal biological function of this potent p53B? To address this, we examined p53 isoform expression using tagged GFP-p53A or mCherry-p53B in genomic BAC transgenes. GFP-p53A localized to a discrete subnuclear body in almost all somatic and germline cells. In contrast, mCherry-p53B was rarely expressed in somatic cells, but was highly expressed in the female germline, co-localizing with GFP-p53A to subnuclear bodies in all nurse cells and oocytes. Importantly, our initial analysis of isoform specific mutants supports that p53B has a function in the female germline. Our results are consistent with other evidence that the function of p53 in the germline likely predated its function in the soma. Defining the germline function of different p53 isoforms will provide insight into what developmental cues may have shaped the ancestral p53 network and the logic of the more complex p53 code in humans.

3. Downstream targets of the Forkhead domain transcription factor Jumeau mediate cardiac progenitor cell specification and division.

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While at least eight Forkhead (Fkh/Fox) transcription factors (TFs) are required for proper cardiac development in mammals and mutations in four Fkh genes have been linked to human congenital heart defects, relatively little was known about the molecular mechanisms or the downstream target genes by which these Fkh TF-mediated cardiogenic functions are brought about. Our prior work had shown that the *Drosophila* Fkh gene *jumeau* (*jumu*) mediates both cardiac progenitor cell specification by regulating the expression of the FGF and Wnt signaling pathways receptors Heartless and Frizzled respectively, and cardiac progenitor cell division by regulating the activity of the Polo kinase. However, the significant enrichment of Fkh TF binding sites in the enhancers of cardiac genes suggested that *jumu* might be utilizing additional downstream target genes to regulate these two cardiogenic processes. Using RNA-sequencing to compare genome-wide transcriptional expression profiles of flow cytometry-purified mesodermal cells from wild-type and *jumu* loss-of-function embryos, we detected 1,272 putative *jumu* targets, i.e. genes exhibiting significant differential expression in *jumu* mutants compared to wild-type. Preliminary phenotypic analysis of a prioritized subset of these downstream targets with amorphic and hypomorphic mutations has enabled us to identify an additional gene mediating cardiac progenitor specification, eight additional genes mediating cardiac progenitor cell divisions, and yet another gene required for the cytokinesis of cardiac progenitor cells.

4. Role of *drop dead* in spermatogenesis

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The *drop-dead* (*drd*) gene encodes a proposed acyltranferase and is expressed in epithelial cells. Severe mutations in this gene lead to early adult lethality, gut dysfunction, and female sterility. No effects on male fertility have previously been reported for *drd* mutations, despite an expression pattern of *drd* in the testis that resembles that of somatic cyst cells. A deficiency screen for enhancers of the mild allele drd^{G3} identified a deficiency, *Df(2L)drm-P1*, that not only enhances adult lethality but also causes synthetic male sterility. To investigate the cause of this sterility, and to determine whether *drd* mutants also show similar defects, we characterized abnormalities that occur during spermatid individualization and transfer of mature sperm to the seminal vesicle. Total and abnormal individualization complexes (ICs) were quantified after staining testes of various *drd* mutants with phalloidin. All *drd* mutants showed a reduced number of total ICs compared to wildtype (WT), as well as an increased fraction of abnormal ICs. To assess if these abnormal ICs affect fertility, aged males with RNAi knockdown of *drd* in the somatic cyst cells of the testis were crossed to WT females to quantify the number of progeny. This resulted in no difference, suggesting *drd* plays a minor or redundant role in

the testis. To address the cause of synthetic sterility observed in *drd^{G3}; Df(2L)drm-P1/+* males, we used fluorescently labeled sperm, *dj-GFP*, and observed that mature sperm fail to transfer to the seminal vesicle. In order to narrow down the gene in the deficiency responsible for this phenotype, we used a smaller deficiency, *Df(2L)BSC292*, that lies within *Df(2L)drm-P1* in the *drd^{G3}* background to look at their sterility. 65% of the males are sterile, suggesting that the gene responsible for the synthetic sterility lies near the breakpoint of *Df(2L)BSC292*. These data demonstrate a previously unknown role of *drd* in male fertility and suggest that the gene functions both during spermatid individualization and during sperm transfer to the seminal vesicle. Supported by NSF grant IOS-1355087.

5. Capicua's Role in the Regulation of Tissue Regeneration

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Certain organisms exhibit the capacity to regenerate damaged fragments of their body following injury. The process of tissue regeneration varies between organisms and encompasses the ability to restore original morphology and function. The Receptor Tyrosine Kinase (RTK)/Ras pathway plays a predominant role in inducing tissue regeneration and has been shown to be up-regulated in a multitude of regenerative contexts such as the *Drosophila* intestine and rat liver (Jiang et al., 2011; Chen et al., 2013). Capicua (*cic*) is a transcription factor that acts as a tumor-suppressor gene (Jiménez et al., 2012). In the *Drosophila* intestine, *cic* is down-regulated by Ras signaling, which leads to increased proliferation of stem cells during regeneration (Tseng et al., 2007; Jin et al., 2015). However, the exact role of *cic* in multiple regenerative contexts isn't fully understood. We use a novel genetic ablation system to induce damage and regeneration in the *Drosophila* wing imaginal discs (Smith-Bolton et al., 2009). The contribution to regeneration of certain genes can be better understood by observing the regenerative capacity of mutants of such genes using this system. Results show that *cic* mutants have improved regenerative capacity following ablation. Preliminary data suggests that following ablation *cic* mutants have more time to regenerate and show increased expression of the cell cycle regulator MYC. Although further experimentation is needed, it appears that *cic* may play an inhibitory role in wing imaginal disc regeneration.

6. Investigating the role of metabolic regulation during an immune response

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A functional immune response is essential for the survival of all living organisms. The fruit fly *Drosophila melanogaster* is a well-established model for studying host immunity as well as metabolic regulation during an immune response. Fruit fly larvae, in nature, are commonly infected by parasitoid wasps whose survival is dependent on the successful parasitization of their host. The immune response that the fly elicits toward the wasp egg is a primitive yet effective process that is energetically costly for the host. Preliminary evidence using RNA interference (RNAi)-mediated knock down of PVR, a cell-surface tyrosine kinase receptor, has shown to have an effect on the fruit flies' ability to regulate energy storage during an infection, which in turn affects post infection survival. Furthermore, microarray studies have shown elevated expression of PVR's specific binding ligands, PVF 1-3, at differing time points during the immune response. This suggests that PVR and its ligands function as a regulatory pathway throughout the course of infection. Further genetic manipulation of the PVR pathway in infected and uninfected flies will allow us to determine how metabolic processes contribute to the immune response and host survival.

7. Using the D. melanogaster accessory gland as a model for prostate cancer

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The D. melanogaster accessory gland (AG) is a secretory epithelium functionally analogous to the mammalian prostate. The use of D. melanogaster as a model for prostate cancer is advantageous due to its rapid life cycle and facile genetic manipulation. Prostate cancer risk is age-associated and the human prostate often grows and becomes hypertrophic with age. We measured the normal, physiological level of cell cycling in the adult D. melanogaster accessory gland along with tissue morphogenesis and growth. Similar to the human prostate, we find evidence of continued growth and age-associated cellular hypertrophy in the accessory gland. We developed a FACS protocol to analyze DNA content and examine aneuploidy/hyperploidy in this tissue and we can distinguish between the two epithelial cell types of the gland, the “main” cells and the secretory “secondary” cells. We have confirmed that a gene associated with prostate cancer, YAP (Yki) generates a hyperplastic mass in the accessory gland when a gain of function allele is expressed. To our knowledge this is the first demonstration of a tumor-like structure in the accessory gland of D. Melanogaster.

8. The Role of Kinase Fusion DNAJB1-PRKACA in Fibrolamellar Hepatocellular Carcinoma

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Kinase fusion has been detected in a variety of cancer types. Approximately 3% of cancers are associated with different kinase fusions, including fibrolamellar hepatocellular carcinoma (FL-HCC). FL-HCC is a rare type of liver cancer that predominantly occurs in the teenage population without any previous liver disease history. It is aggressive and currently surgical resection remains the only effective therapeutic. DNAJB1-PRKACA fusion is detected in at least 80% of FL-HCC cases and is considered a potential oncogenic factor. However, the causality between DNAJB1-PRKACA fusion and FL-HCC is not yet established. This study aims at investigating the function of DNAJB1-PRKACA fusion in FL-HCC oncogenesis using Drosophila and murine in vivo models. Human DNAJB1-PRKACA (hDNAJB1-PRKACA), when over-expressed in Drosophila eye progenitor cells, induced proliferation and differentiation phenotypes, including decreased eye size and abnormal eye shape. Genetic and pharmaceutical inhibition of PKA activity rescued the eye phenotype, while apoptosis inhibitor over-expression exaggerated the tumor eye phenotype. This eye phenotype was used to elucidate potential therapeutics such as PKA inhibitors and several other compounds. hDNAJB1-PRKACA over-expression in fly intestinal stem cells perturb histone-chromosome interaction and caused severe tumors in several cases. For modeling the potential oncogenic fusion in murine hepatocytes, CRISPR/Cas9 genome engineering was used to recreate the murine chromosomal deletion equivalent to that found in FL-HCC patients. gRNAs were designed to target mouse DNAJB1 and PRKACA introns and tested for their editing efficiency. Co-transfection of a pair of effective gRNAs successfully generated a 360kb chromosomal deletion on chromosome 8 in mouse hepatocytes. Multiple single cell clones were isolated, first characterized for their in vitro proliferation properties, and further inoculated subcutaneously in vivo to monitor their oncogenicity.

9. Transport of the SERCA virulence factor through parasitoid wasp venom

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Heart failure afflicts between 5-6 million adults in the U.S. and an estimated 26 million people worldwide, costing billions of dollars each year to treat. Heart failure is characterized by the inability of the heart to pump a sufficient volume of oxygenated blood through the vascular system due to a compromised excitation-contraction (EC) coupling in myocardial cells. Although deficiencies in EC coupling aren't a direct cause of death, the prolonged diminished output of the heart can be fatal. The Sarco/endoplasmic reticulum calcium ATPase (SERCA) has been shown to play an important role in EC coupling. In heart failure studies, SERCA expression was significantly reduced leading to atypical calcium cycling and EC deficiency. We are interested in using the host-parasite relationship between *Ganaspis sp.1* wasps and *Drosophila melanogaster* flies as a model to study SERCA activity. The parasitoid wasp venom contains SERCA, which is transported into the immune cells of *D.* melanogaster larvae, and acts to inhibit the immune response. We believe that the wasp venom SERCA has evolved to infiltrate host immune cells and disrupt calcium signaling, leading to an immune deficiency. Currently, we are characterizing how wasp SERCA is transported through the venom and targeted to the immune cells.

10. The Role of the *Dop* Kinase in Hedgehog Signaling and Cell Morphology

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Crucial to healthy development is the collection of proteins involved in Hedgehog (Hh) signaling. Hh signaling has a vital role in pattern and tissue development in embryos as well as physiology later in life in both vertebrates and invertebrates. Although a considerable amount of information is already known about this pathway, there are significant aspects of the pathway that are not understood. To identify new components of the pathway, an RNAi screen was performed in a genetically sensitized background. Knockdown of the gene encoding the *dop* kinase led to decreased Hedgehog pathway activity. In collaboration with the Muller lab, we showed that clones mutant for *dop* had decreased expression of the Hedgehog target gene *ptc*. Unexpectedly *dop* mutant clones failed to respect the compartment boundary and appeared to be invasive. *Dop* mutant clones have unusual morphology and send out actin filament containing projections. The clones also induced atypical folds in the wing imaginal disc epithelium. To date I have used static images of fixed Drosophila wing discs to examine the relationship between dop and the actin containing projections, but this will not reveal how these changes progress. Live-cell imaging will be able to determine exactly how actin is reorganized in the *dop* mutant clones and how this leads to the buckling of the disc epithelium. Ultimately, a better understanding will help further knowledge on Hh signaling and thus can be used to counteract diseases and defects associated with this pathway.

11 The Notch signaling pathway specifies cardiac cell subtypes by regulating the expression of different pericardial genes through distinct permissive and instructive mechanisms.

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The development of a complex organ involves the specification and differentiation of diverse cell types constituting the organ. The *Drosophila* heart is comprised of two major cell types: contractile cardial cells (CCs) that constitute an inner tube and pericardial cells (PCs) that form a sheath surrounding the CCs. Our previous work showed that binding sites of Suppressor of Hairless [Su(H)], an integral transcription factor in the Notch signaling pathway, were enriched in the enhancers of genes specifically expressed in the PCs. Furthermore, by using *cis*- and *trans*- assays with enhancer-reporter constructs for a PC-specific gene, *Holes in muscle* (*Him*), we had demonstrated that Notch signaling activates *Him*

expression in PCs in a permissive manner: in the absence of Notch signaling, Su(H) forms a repressor complex with co-repressors and binds to the *Him* enhancer, repressing its transcription; upon alleviation of this repression by Notch signaling, *Him* transcription is activated. Here, once again using enhancer-reporter constructs, we provide preliminary data showing that in the case of a different PC-specific gene, *Zn finger homeodomain 1* (*Zfh-1*), Notch signaling activates *Zfh-1* expression in PCs in a distinctly different, instructive manner: simply alleviation of repression by the Su(H) repressor complex is no longer sufficient to activate transcription; in the case of *Zfh-1*, upon Notch signaling, the Notch intracellular domain must bind with Su(H) to change the Su(H) complex bound on the *Zfh-1* enhancer from a repressor to an activator complex. Collectively, these data show how the same feature, enrichment of Su(H) binding sites in the enhancers of PC-specific genes, can be utilized by two distinct mechanisms to contribute to the same overall goal: the specification and differentiation of pericardial cell types by activation of the pericardial gene program.

12. Quantitative analysis of Ca^{2+} signaling downstream of Decapentaplegic signaling in the *Drosophila* wing imaginal disc

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Organ development is driven by a set of patterned inductive signals. However, how these signals are integrated to coordinate tissue patterning is still poorly understood. Calcium ions (Ca^{2+}) are critical signaling components involved in integrating many upstream signals. Ca^{2+} signaling encodes a significant fraction of information in cells through both amplitude and frequency-dependent regulation of transcription factors and key regulatory enzymes. Recently, we have reported specific spatiotemporal patterns of Ca^{2+} signatures in developing wing discs. This has been enabled with a new neural-network-based approach for registration of tissues with oscillatory signals that frequently move during imaging, and a pipeline for spatiotemporal analysis of intercellular Ca^{2+} oscillations. As a specific test case, we further demonstrated that the morphogen pathway, Hedgehog, controls frequencies of Ca^{2+} oscillations uniformly in the tissue and is required for spatial patterning of oscillation amplitudes. Here, we report efforts to expand the analysis to comprehensively map the relationship between other morphogenetic signals such as the bone morphogenetic protein Decapentaplegic and Ca^{2+} transients in developing epithelia. These mapping experiments will provide an important dataset for inferring signaling interactions during organ growth and morphogenesis.

13. Exploring the relationships between nuclear actin, prostaglandins, and the cell cycle

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Actin is not simply a cytoskeletal component, but localizes to and functions within the nucleus. While cell culture studies have implicated nuclear actin in regulating transcription, DNA damage repair, and chromatin organization, the roles of nuclear actin in development remain unknown. Previous research in the Tootle lab has shown that nuclear actin is dynamically regulated during *Drosophila* oogenesis. During oogenesis, follicles made up of ~1000 somatic follicle cells and 16 germline cells, consisting of 15 nurse cells and 1 oocyte, progress through a series of 14 morphological stages, from the germanium to Stage 14 (S14). We find that germline expression of GFP-Actin induces nuclear actin rods in the nurse cells during Stages 5-9. Similarly, using an antibody to actin (C4), we find that a subset of the nurse cells exhibit structured or blobby nuclear actin beginning around S5 and decreasing until S9. Using both GFP-Actin rod formation and anti-Actin C4 staining we identified prostaglandins (PGs) as a regulator of nuclear actin during oogenesis. PGs are lipid signals made downstream of cyclooxygenase (COX) enzymes and regulate many processes in the body, including reproduction and fertility. In *Drosophila*, Pxt is the COX-like enzyme and regulates follicle maturation and the actin cytoskeleton. We

find that loss of Pxt leads to an increase in the number of nuclear actin rods being formed when GFP-Actin is expressed. It also results in increased levels of endogenous structured nuclear actin (C4) in the nurse cells. These data lead us to propose that prostaglandins negatively regulate nuclear actin. We next sought to uncover the functions of nuclear actin. Given the unusual appearance of the structured nuclear actin, we reasoned that identifying where in the nucleus nuclear actin localizes to would provide functional insight. We find that the structured nuclear actin resides within the nucleolus. Interestingly, prior studies have shown that PGs regulate nucleolar structure during oogenesis, leading us to speculate that PGs regulate nuclear actin to control nucleolar structure and function. As the structure of the nucleolus is regulated by the cell cycle, and the nucleolus is implicated in sequestering cell cycle regulators, we next examined the relationship between cell cycle and nuclear actin. The nurse cells undergo asynchronous endocycles that are controlled by Cyclin E. Labeling follicles with an anti-Cyclin E antibody shows pulses of Cyclin E in the nurse cells similar to the pulses of C4 nuclear actin. Analysis of Cyclin E and C4 double staining shows some overlap in the nurse cells, with the nurse cells having the highest C4 nuclear actin staining exhibiting high Cyclin E. This finding led us to examine how altering the cell cycle affects nuclear actin. Previous research showed that hypomorphic loss of Cyclin E during oogenesis results in a longer S phase. We find that RNAi knockdown of Cyclin E affects DNA replication, as many nurse cells remain polytene through late stages of oogenesis. We also see an increase in structured nuclear actin in the nurse cells. The morphology of the nuclear actin is also distinct, raising the possibility that nucleolar structure is perturbed. These results suggest a relationship between the cell cycle and nuclear actin. Future studies will further explore the interplay between nuclear actin, the cell cycle, the nucleolus and PGs. Ultimately, these studies are expected to significantly advance our understanding of the in vivo regulation and functions of nuclear actin.

14. Determining the function of the transcription factor Zelda in driving neural stem cell fate

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Stem cells are unique in that through asymmetric divisions they are able to both self- renew and generate differentiated progeny. Maintaining the precise balance between self-renewal and differentiation is necessary for proper development and when misregulated can lead to tumors. Transcription factor regulatory networks are essential for controlling stem cell fate and differentiation following division. To better understand the mechanisms by which transcription factors control this balance, we are studying stem-cell populations in both the early embryo and neural lineages of *Drosophila.* We have previously demonstrated that Zelda (ZLD), a zinc finger transcription factor, is necessary to activate transcription and initiate embryonic development. ZLD activity must be precisely regulated, as too much or too little is lethal to the embryo. ZLD is also expressed in the developing central nervous system. Recently, we showed that this expression is limited to the neural stem cells (neuroblasts), and overexpression of ZLD leads to increased neuroblast formation. These data suggest that ZLD activity in the stem cells may facilitate stem-cell maintenance. Misexpression of ZLD in the partially differentiated progeny of the neuroblasts also leads to extra neuroblasts. Thus, similar to the early embryo, ZLD activity must be precisely regulated during neuronal differentiation. We are currently investigating how ZLD expression is restricted to the neural stem-cell population following asymmetric division. Preliminary data suggest that the Trim-NHL protein Brain tumor (Brat) may regulate ZLD levels both in the early embryo and in the neural stem cell lineage. Together, our studies of ZLD in the early embryo and neuroblasts will advance our understanding of how transcriptional and post- transcriptional mechanisms precisely regulate stem cell fate.

15. A *Drosophila* model of bacteremia
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Blood infections (termed bacteremia) are becoming increasingly more common as invasive medical procedures become a more regular part of modern medicine, and opportunistic bacteria take advantage of the lowered defenses of patients to use them as hosts. The most common pathogen in these hospital related bacteremia cases is *Staphylococcus epidermidis*, a nearly ubiquitous member of human skin flora. Once in the blood stream, *S. epidermidis* can cause a myriad of issues, most notably colonization and infection various tissues throughout the body, such as the heart. This can lead to a systemic innate immune response termed sepsis. Sepsis is a severe and possibly fatal condition characterized by over activation of the inflammation response that can persist long after the bacteria has been eradicated. The inner molecular workings of bacteremia and sepsis progression have yet to be uncovered. Here, we have found that laboratory strains of *Drosophila melanogaster* are susceptible to a bacteremia-like phenomenon in which opportunistic bacteria are consumed and then invade the hemolymph through the cells of the gut. We have isolated a strain of *S. epidermidis* from infected flies that seems to be the cause of infection. We now hope to use this strain along with *D. melanogaster* to create a new model for the study of bacteremia and sepsis progression.

16. Interactions Between the COG Complex and ATP7A in Neurodegenerative Disease
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Menkes disease is an X-linked neurodegenerative disease caused by a defect in copper transport. Such defects can come from mutations in the ATP7A copper transporter gene. ATP7A mutations result in abnormal distribution of copper, leading to reduced copper in the brain and other tissues. One of the protein complexes found to interact with the ATP7A gene is the Conserved Oligomeric Golgi (COG) complex. For this study, we are modeling the interactions between the COG complex and ATP7A in the *Drosophila* nervous system. Since ATP7 is a copper transporter, we wanted to test how manipulating COG1, COG5, and COG8 affects ATP7's ability to respond to copper. We have performed experiments with the COG1 mutants crossed with ATP7 over-expression flies to determine how loss of COG1 influences the viability of ATP7 over-expression flies. We found that the overexpression of ATP7 resulted in reduced viability. COG1 mutants that also overexpressed ATP7 showed no viability, whereas COG1 mutants showed a reduced phenotype. We also found that inhibiting other members of the COG complex show a reduced viability phenotype. Similar results were found during our copper experiments testing COG5 and COG8. We have found that loss of either COG5 or COG8 cause increased sensitivity to copper as compared to the controls. Also, the combination of both ATP7 over-expression and COG5 inhibition and ATP7 over-expression and COG8 inhibition both showed a significant increase sensitivity copper compared to the control. The combination also showed a slight reduction in copper sensitivity as compared to both inhibition of COG5 or COG8. We are currently are testing the interactions of other members within the COG complex.

17. Differential roles of calcium signaling channels on epithelial tissue morphogenesis and homeostasis
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The robust formation of organs during development depends on the careful regulation of cellular processes such as cell adhesion, mechanical stiffness of the cell membrane, and internal pressure to create the three-dimensional tissue-scale architecture. A sophisticated communication system coordinates these developmental processes. Many birth defects and diseases occur when this communication system breaks down. In particular, calcium ions play critical roles in regulation of cell mechanics during organ development. However, the regulation and function of the core calcium-based signaling systems and its role in regulating tissue mechanics are still poorly understood. Here we tested

a hypothesized mechanism of stress dissipation mediated by Ca^{2+} during tissue development. To do so, we perturbed Ca^{2+} concentrations in cells genetically through perturbation of IP_3R channels, which control flux of Ca^{2+} from endoplasmic reticulum stores into the cell cytoplasm and SERCA channels, which remove Ca^{2+} from the cytoplasm into the ER. We show that both increased and decreased cytoplasmic Ca^{2+} concentrations result in decreased apical E-cadherin localization and tissue shape defects in the wing disc pouch of *Drosophila melanogaster* 3rd instar larva. Furthermore, tumor-like overgrowth was observed in late stage wing discs when SERCA is inhibited. In contrast, inhibition of store operated calcium entry through Stim or Orai did not lead to the tumor-like phenotype. Together these results map specific components of the core Ca^{2+} signaling toolkit to regulation of tissue morphogenesis and homeostasis.

18. Circadian environmental cues modulate aging in *Drosophila melanogaster*

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In recent years it has been demonstrated sensory perception modulates aging. Manipulations of only a few sensory neurons can improve health and increase lifespan. However, the manner and modalities by which sensory neurons orchestrate changes throughout the organism to promote beneficial effects on health and longevity remain unknown. One largely unexplored perceptual system in the context of longevity is how we perceive time. Animals exhibit daily behavioral and physiological rhythms, but what happens when these endogenous rhythms do not align with environmental events and time cues? We used *Drosophila melanogaster* to study how circadian rhythms and environmental time cues interact to influence longevity. Perturbations of the circadian system in humans lead to increases in obesity, diabetes, and metabolic syndrome. This is of particular importance as the number of shift workers, a group susceptible to circadian disturbance, is on the rise. Here we test the hypothesis that greater environmental synchrony contributes to longevity and health-span.

19. The role of CG17352 in Drosophila photoreceptor homeostasis

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Much progress has been made towards unraveling the pathways involved in the generation and maintenance of visual systems. Problems with the molecular players in these pathways can lead to retinal degeneration, and identifying and characterizing these molecules is key to understanding the mechanisms for specification and maintenance of the retinal structure. Our lab previously identified CG17352 as a putative target of Glass, a zinc-finger transcription factor that mediates photoreceptor differentiation through a network of genes. Photoreceptor cells in CG17352 mutants develop normally, but degenerate in adult flies in a light dependent manner. This phenotype suggests a role for CG17352 in maintaining photoreceptor cells. We will present data from sets of experiments exploring the role of CG17352 in photoreceptor maintenance.

20. TEL-mediated dysregulation requires the activity of higher order polymers

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In order for a cell to survive and function, it must properly regulate the transcription of its genes. Many human cancers are driven by mutations in transcription factors(TFs), yet how such mutations dysregulate gene expression is not well understood. TEL/ETV6 is an evolutionarily conserved polymerizing TF that normally regulates blood development in mammals. Polymerization, which is mediated by the Sterile Alpha Motif (SAM) domain, is essential to TEL's ability to regulate gene expression during development. Chromosomal translocations that fuse the polymerizing SAM domain of TEL to the DNA binding domain of another TF, in essence introducing a novel polymerizing TF, have been shown to lead to some forms of leukemia. How these converted polymerizing TFs disrupt endogenous transcription is not well understood nor is it known how the strength of polymerization modulates transcriptional output during either normal or oncogenic development. To answer these

questions, I am developing a model system, reminiscent of the introduction of a novel TF through a mutational event, in which mammalian TEL is overexpressed with a series of biochemically characterized point mutants that reduce SAM-SAM affinity in the developing eye of *Drosophila melanogaster*. The consequences with respect to gene expression, cell fate specification, and overall tissue patterning are then assessed. I have shown a positive correlation exists between the SAM-SAM interaction strength and the severity of disruption in the normally well-organized eye. Using this system, it has also become possible to determine what aspects of polymerization, polymer length or protein-protein affinity, contribute most to TEL-mediated dysregulation. Normally a difficult question to address, as increased binding affinity induces longer polymers, it is possible to restrict TEL to dimers and then independently modulate the SAM-SAM affinity. Even at their highest affinity, I have found that dimers are insufficient to recapitulate full length TEL activity, suggesting that higher order polymers are required for TEL- mediated dysregulation. Future work will attempt to elucidate the molecular mechanism for polymerization-dependent TEL-mediated regulation and dysregulation of gene expression by examining changes in target gene expression levels and in TEL chromatin occupancy in the polymerization mutant series.

21. The adaptor protein, Dreadlocks, is essential for normal ring canal expansion and membrane stability in the developing egg chamber

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Infertility is a widespread problem, the root of which is still poorly understood. Current treatments are invasive and met with low success rates. Achieving a better understanding of the causes of infertility is essential to the development of better treatment. One possible origin of infertility stems from errors in gametogenesis. In the fruit fly, eggs develop from multicellular structures called egg chambers; the egg chamber is composed of a cluster of 16 germ cells, which consist of fifteen nurse cells and one oocyte, surrounded by a layer of epithelial cells. The germ cells are connected by intercellular bridges called ring canals. Ring canals are formed through incomplete cytokinesis and allow for the cytoplasmic transfer of materials from the nurse cells to the oocyte. Towards the end of oogenesis, the nurse cells empty their cytoplasmic contents through the ring canals and into the oocyte during nurse cell dumping. This process is imperative as it provides the oocyte with the necessary nutrients to become a viable egg. Nurse cell dumping depends on the incredible stability of the ring canals to withstand the pressure associated with bulk cytoplasmic transfer. Thus, our lab is interested in the proteins involved in ring canal formation, expansion, and stability. We have identified a novel role for the SH2/SH3 adaptor protein, Dreadlocks (Dock) in regulating ring canal expansion and membrane stability in the germline. Depletion of Dock leads to ring canal over-expansion. Interestingly, over-expression of Dock leads to membrane destabilization and the formation of highly multinucleate nurse cells. Future studies will characterize the mechanisms by which Dock regulates ring canal expansion and membrane stability.

22. Cloning of Muscular Dystrophy Genes in Drosophila

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Muscle related diseases are linked to many different genes though how these genes interact with each other to cause a disease are not fully understood. One set of genes involved in a variety of muscle diseases code for proteins that form a protein quality control complex called the Chaperone Assisted Selective Autophagy (CASA) Complex. The CASA complex consists of the molecular chaperones Hsc70 and HspB8 and the co-chaperone BAG-3 (starvin in flies). We find that the CASA complex interacts with the p38 MAPK to regulate muscle aging. Due to a lack of good antibodies to the members of the CASA complex and p38 MAPK, we are cloning the various members and their isoforms to make tagged proteins that can be easily tracked in the cell and be used for biochemistry analysis. To experiment on these specific sequences of genes, the first step is to isolate your gene of interest and purify. Transforming your RNA extracted to cDNA is important to create a more stable structure and prevent mutations or alterations in your sequence. Next, you must amplify your DNA with PCR, purify

with ethanol precipitation or gel purification, transform or clone your DNA into cells, and finally, do plasmid preparation on the DNA. We then sequence the plasmids to make sure the DNA was correctly inserted and then use the correct plasmids for making transgenic flies.

23. Dystrophin's structure, subcellular organization, and roles in development.
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Mutations in actin-membrane linker protein Dystrophin (Dys) are the cause of Duchenne Muscular Dystrophy (DMD). Detailed understanding of Dys protein's basic cellular and developmental roles is important for designing effective muscular dystrophy therapies. In Drosophila, Dys mutations are viable and produce two visible phenotypes: wing crossveins are detached from the longitudinal veins, and in oogenesis the developing eggs fail to elongate. A GFP protein trap in the endogenous Dys locus shows that Dys is localized at the basal side of epithelia such as the egg chamber follicle cells, where it makes a plane-polarized striated pattern. Dys-GFP also accumulates on membranes of the egg chamber nurse cells, especially near the actin ring canals. However, in both cases Dys-GFP maintains a pattern distinct from the nearby actin bundles. Loss of Dys leads to failure of circumferential banding of f-actin in the follicle cells, but promotes excess cell surface projections. *Dys-RNAi* eliminates Dys-GFP, confirming both constructs function as expected. Germline RNAi against Dys does not trigger the typical *Dys* phenotypes, indicating Dys is instead required in the follicle cells and/or ovary muscle. The beta-Heavy spectrin ("betaH" encoded by *karst*) is structurally related to Dys, and so we checked for redundancy between them. betaH, Dys double mutants have severe ovary defects and high lethality, unlike the single mutants, suggesting the two spectrin family members overlap in their developmental functions. The sequence of fly Dys was re-analyzed in light of newer structural data from the mammalian protein; we find that Dys is structurally well-conserved except that spectrin repeats 14-15 (of 24) are degenerated, possibly forming an extra hinge region.

24. Identification and analysis of JAK-STAT pathway regulator genes in Drosophila immunity
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Drosophila melanogaster larvae are commonly infected by parasitoid wasps and in response, mount a robust cellular immune response against the parasitoid egg. This immune response involves fly immune cells called hemocytes and culminates in the melanotic encapsulation of the wasp egg. However, the regulations of the conserved signaling pathways controlling *D. melanogaster* immunity are not completely understood. To seek a better understanding of the regulators of one such signaling pathway, JAK-STAT, we are using an autoimmune D. melanogaster mutant, *tumor Suzuki* (*tuSz*). These flies have a gain of function mutation in the JAK-STAT pathway, resulting in hyperactive immune response and the encapsulation of their own fat body tissue. We have identified 7 candidate genes that we predict are involved in fly immunity and the JAK-STAT pathway. We used RNAi to knock down the candidate genes in specific hemocytes types in tuSz flies, to test their ability to modify the gain of function JAK-STAT signaling phenotype. Based on our current results, we have found that several of the candidate genes are negative regulators of the JAK-STAT pathway and that one of the candidate genes is a downstream effector of the JAK-STAT pathway, ultimately influencing the *D. melanogaster* cellular immune response. Further, we performed wasp infection studies to test our hypothesis that these candidate genes are important for the cellular encapsulation of parasitoid eggs. Our results indicate that controlled regulation of the JAK-STAT pathway is crucial in order to keep the immune response in check. Analysis of our current findings provides valuable insight into the role played by these genes in JAK-STAT pathway, an important aspect of fly immunity.

25. Metabolic Dysregulation following Traumatic Brain Injury in Drosophila melanogaster

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Traumatic Brain Injury (TBI) is a major cause of disability and death worldwide, with numerous lasting effects including cognitive decline, memory impairment and worsened emotional functioning. Many of these effects are likely results of secondary cellular injury following the primary mechanical insult. Data from clinical studies of TBI strongly suggest evidence of metabolic dysfunction, with hyperglycemia recognized as an independent predictor of mortality, and a higher mortality rate of diabetics following TBI compared to matched controls. Understanding secondary cellular injury following the primary insult in TBI is crucial both for management and prevention of mortality in the acute setting, as well as ameliorating it's effects over the long term in TBI survivors. While great progress has been made in characterizing the metabolic state of an organism following TBI, there is still much to be understood in regards to identifying specific cellular pathways and substrates involved in this dysregulation.
Our lab has developed a *Drosophila melanogaster* model of TBI that involves a spring-based High-Impact Trauma (HIT) device to inflict mechanical injuries on the flies, and results in a period of temporary incapacitation, ataxia, and neurodegeneration, which indicates it does inflict primary injures to the brain. I am using this model to characterize the change in the metabolic state of the cell immediately following TBI, as well as to elucidate mechanisms that result in disrupted energy homeostasis. This is accomplished by determining the mortality index at 24 hours (MI_{24}) following TBI after exposing flies to a variety of diets and substrates involved in glycolysis, as well as performing assays that determine levels of metabolic activity in fly brains post-TBI including ATP concentration and Lactate:Pyruvate ratios. Our approach takes advantage of the larger sample sizes and genetic variability of the *Drosophila* model by characterizing metabolic dysregulation both in our w^{1118} lab strain, as well as previously identified TBI sensitive and resistant fly lines, to highlight the role genetic background plays in the metabolic crisis post-TBI. Future studies will include a candidate gene approach utilizing RNAi to elucidate cell-specific critical metabolic pathways that may contribute to neuronal cell death following TBI, as well as an unbiased forward genetics screen to identify genes contributing to TBI sensitive or resistant phenotypes. Recent data in our *D. melanogaster* model of TBI is already offering intriguing insights into the possible mechanisms behind hyperglycemia and it's effect on post-TBI mortality. Similar to mammalian and clinical models, our data demonstrates a transient period of hyperglycemia as well as a decrease in ATP levels in the first 24 hours post-TBI. We have also discovered that feeding flies a high sugar diet post-TBI significantly increases their MI_{24} and correlates with the concentration of glucose in the hemolymph. We have further been able to generate a positive effect on the MI_{24} by blocking certain hyperglycemia-induced pathways, such as the the polyol pathway, through feeding the flies epalrestat, an inhibitor of aldose reductase. Potential outcomes of these studies and future experiments could result in a better understanding of the key players involved in cellular dysregulation following TBI, as well as present an opportunity to identify novel targets for metabolic therapies.

26. Do cells select against a high frequency of mitochondrial DNA mutations?

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Mitochondria arose due to a merging event between two independent life forms. After nearly two billion years of co-evolution, these organelles still retain their own greatly reduced genome, encoding proteins essential for generating energy. As mitochondrial DNA (mtDNA) replicates over an organism's lifetime, it acquires many mutations, a process that has been linked to aging and neurodegenerative disorders, such as Parkinson's Disease. To combat the deleterious effects of damaged mitochondria, cells selectively target them for autophagic destruction through the PINK1/Parkin pathway. While we know that damaged mitochondria are selectively degraded, it is unclear whether there are mechanisms in

place to select against potentially harmful mutations in the mitochondrial genome. I am therefore studying this question by overexpressing Parkin in *Drosophila* strains that generate high levels of mtDNA mutations. Previously published data suggests that the PINK1/Parkin pathway and autophagy play a role in decreasing mitochondrial DNA mutations. However, my findings indicate that overexpression of Parkin does not greatly decrease the number of mtDNA mutations. This may indicate that the PINK1/Parkin pathway does not select against mutated mitochondrial DNA in somatic tissues. Continuation of this research will provide critical insight into the mechanisms by which harmful mtDNA mutations rise in abundance and cause disease.

27. The role of Forkhead domain transcription factors and their downstream targets in mediating proper positioning of cardiac cells

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The development of a complex organ requires the specification of appropriate numbers of each of its constituent cell types as well as the correct positioning of these cell types within the organ. Our previous work on *Drosophila* embryonic heart development had shown that the Forkhead (Fkh/Fox) domain transcription factors Checkpoint suppressor homologue (CHES-1-like) and Jumeau (Jumu) determine the correct number of different cardiac cell types by regulating the division of cardiac progenitor cells through a Polo-dependent pathway. Here we show that *CHES-1-like* and *jumu* are also required for the correct positioning of these cardiac cell types: loss-of-function mutations in or RNA interference knockdown of either gene results in the misalignment and incorrect locations of both cardial and pericardial cells within individual metameric repeating units called hemisegments. Since defective cardiac progenitor cell divisions in *CHES-1-like* and *jumu* loss-of-function mutants frequently result in individual hemisegments having different numbers of cardiac cells than their partners across the dorsal midline, we initially examined this asymmetry as a possible steric cause of incorrect positioning. Our statistical analyses revealed that steric constraints imposed by the differing number of heart cells in contralateral hemisgments are not sufficient to explain all of the observed defects in cardiac cell positioning: statistically significant increases in the number of incorrectly positioned cardiac cells are also observed in Fkh mutants compared with wild-type embryos when only members of contralateral hemisegment pairs having the same number of each cardiac cell type are compared. In order to find additional downstream targets which might be utilized by *CHES-1-like* and *jumu* to bring about correct positioning, we next compared genome-wide transcription expression profiles of purified mesodermal cells from wild-type embryos and embryos lacking functional copies of *CHES-1-like*, *jumu*, or both Fkh genes. We detected 2,131 putative Fkh targets, i.e. genes exhibiting significant differential expression in single or double Fkh mutants compared to wild-type. Our preliminary phenotypic analysis of a prioritized subset of these downstream targets suggests that the Fkh transcription factors bring about the correct positioning of cardiac cell types by restricting the expression of *G protein gamma 1* (*Gγ1*): *CHES-1-like* and *jumu* functions in a mutually redundant manner to repress *Gγ1* expression levels, while ectopic overexpression of *Gγ1* in the mesoderm phenocopies cardiac cell positioning defects observed in *CHES-1-like* and *jumu* loss-of-function mutants.

28. The Role of Btk29A in Tissue Regeneration

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After sustaining an injury, an organism relies on the complex biological process of regeneration to repair damaged tissue. *Drosophila melanogaster* serves as a good model organism to study the tissue regeneration and repair process due to their genetic manipulability, quantity of progeny, short life cycle, and highly regenerative tissue in the larval stage. We study the regeneration of imaginal wing disc tissue following a genetic ablation procedure. These discs are found in the larval stage of development and contain the necessary information for adult wing formation and patterning. The ablation procedure

uses the GAL4/UAS/GAL80 transcriptional regulatory system to conditionally induce apoptosis via *reaper* in the wing pouch tissues. The amount of regeneration can be measured by observing the procedure's effects on adult wing development and patterning. We identified Btk29A as required for regeneration in a pilot genetic screen of known actin regulators. Btk29A is a non-receptor tyrosine kinase, which plays a part in a number of aspects of Drosophila development, including blastoderm cellularization, male genital formation, and oogenesis. Btk29A phosphorylates two conserved tyrosine residues on the Drosophila ortholog of β-catenin, Armadillo. We hypothesize that Btk29A regulates tissue regeneration by regulating the function of β-catenin. β-catenin regulates cell-cell adhesion by altering the actin organization found in structures between neighboring cells. We hypothesize that maintaining these cell junctions after tissue damage is essential for proliferation and in turn, regeneration. We are working to characterize the allele and have found the regeneration phenotype in multiple Btk29A lines as well as a lack of the pupariation delay usually associated with regeneration phenotypes.

29.Identifying Natural Variation in Midline Axon Guidance Using the Drosophila melanogaster Genetic Reference Panel

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The central nervous system (CNS) midline is an important choice point for many pathfinding axons during neural development. Previous studies have searched for novel regulators using mutagenesis experiments involving a few inbred laboratory strains of *Drosophila melanogaster*. However, no studies thus far have attempted to utilize the polymorphic variation that exists in natural populations to study embryonic axon guidance at the CNS midline. This approach was recently enhanced by the creation of the *D. melanogaster* Genetic Reference Panel (DGRP), which consists of more than 200 isogenic, sequenced strains derived from an outbred population. In the present study, embryos from 67 DGRP strains were stained using one of two antibodies: BP102, which labels all axon pathways, or 1D4, which labels a subset of longitudinal axons that normally do not cross the CNS midline. We identified axon guidance defects in the form of missing commissures or ectopic midline crossovers in 17 out of 67 strains. Different strains varied in the penetrance and expressivity of the observed phenotypes. This observation demonstrates that natural variation exists among genes influencing midline axon guidance in *D. melanogaster.* We are now repeating these experiments using the remaining DGRP strains. In addition, we are utilizing a sensitized genetic background to screen for additional strains with axon guidance defects. In the long-term, this research may provide insight into the complex network of ligands, receptors, and signaling molecules that regulate axon guidance.

30.Calcium-dependent regulation of actomyosin contractility after epithelial wounding

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Wound healing in epithelial cells is crucial to prevent infection in many tissues including the skin, lungs, and stomach. However, there is a poor understanding of how biochemical signals and mechanical forces are coupled to coordinate cellular processes during regeneration. Among their many signaling roles, calcium ions (Ca^{2+}) act to regulate mechanical forces generated by actomyosin contractility after wounding. Here, we quantitatively investigate the impact of Ca^{2+} channel activity on wound healing dynamics in the *Drosophila* wing imaginal disc, a powerful model system to study wound healing and regeneration. Two different Ca^{2+} channels, IP_3R and SERCA, were pharmacologically inhibited in the wing disc and the dynamic response to wounding was measured. Precise laser incisions were made along the edge of the cell membranes of two adjoining cells. The recoil of tricellular junctions connecting the cut cell provides a gauge of relative tissue tension. Additionally, the dynamics of the gap size created by the cut were measured. On short time scales, inhibiting IP_3R, did not significantly impact the recoil of tricellular junctions or growth of the wound margin. Inhibition of SERCA increased

the net recoil of tricellular junctions, consistent with increased actomyosin contractility, but dynamics of the wound margin were unaffected. These data suggest that the effect of Ca^{2+} ion concentration has differential effects on actomyosin contractility and remodeling that require further investigation. Finally, we are applying a previously developed mechanical model of epithelial tissue to quantify recoil dynamics. Many FDA approved drugs are available that manipulate Ca^{2+} homeostasis. Consequently, mechanistic insights into how Ca^{2+} signaling impacts actomyosin dynamics and cell contractility can lead to promising therapeutic targets for improving chronic wound healing outcomes.

31. Drosophila Lamin Acts in Both Motor Neurons and Muscle to Regulate Locomotor Functions

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The nuclear lamina is the underlying matrix of cells responsible for the structure and shape of the cell. One type of intermediate filament that composes the underlying matrix is lamin. Mutations in the human lamin A/C gene are known to cause the premature aging disease Hutchinson-Gilford progeria as well as two forms of muscular dystrophy, (1) Charcot-Marie-Tooth disease which affects motor and sensory neurons, and (2) Limb-Girdle Muscular Dystrophy, which affects the muscle. By using *Drosophila* as a model, we examined to see whether possible mutations in the lamin gene would cause visible phenotypic effects that could possibly be traced back to human diseases. By targeting different tissues, we studied the locomotor behavior of the fly. In particular, we focused on altering lamin in motor neurons and muscle tissue. Targeting different tissues resulted in different effects in the ability to perform simple tasks such as walking and climbing. Visible effects such as decreased movement and decreased climbing ability were observed and the impaired movement greatly increased with age. Targeted muscle tissue had worsened phenotypic effects compared to the neural tissue. We are looking further to see how targeting other tissues will result in the negative effects in locomotive behavior.

32. The Role of the Immunoglobulin Superfamily Protein Dpr11 in the Development of the Neural Circuit for Nociception in Drosophila

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Nociception is the act of sensing and responding to noxious environmental stimuli. This ability is vital to escape injury and for general organismal survival. Preliminary data indicate that the immunoglobulin superfamily protein Dpr11 is expressed in the nociceptive neurons of Drosophila larvae and is important for the generation of the nociceptive response. The goal of this project is to determine the molecular role of Dpr11 in nociceptors. It is our hypothesis that this role is in specifying synapse formation in cIVda neurons, the initial step in the nociceptive circuit.

33. The Aging Gene lamin Is Regulated by the p38 MAPK and the CASA Complex

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The aging of an organism is associated with progressive diseases. These diseases, such as Limb-Girdle Muscular Dystrophy (LGMD), are characterized partly by the formation of protein aggregates in the cells. One of these cellular mechanisms which regulates aggregation and protein homeostasis in a cell is Classical Assisted Selective Autophagy (CASA). The CASA complex consists of Hsc70, HspB8, and starvin (stv/BAG-3). We find that the p38 MAPK regulates protein aggregation through the CASA complex as the fly ages. However, the targets of p38 MAPK and the CASA complex are unknown. In order to identify possible targets of p38 MAPK and the CASA complex, we have performed quantitative proteomics. Interestingly, one these targets is lamin, a protein that makes up the nuclear lamina, part of the nuclear envelope. Another interesting aspect to lamin is that mutations in the gene cause LGMD

along with Hutchinson-Guilford Progeria, a disease related to rapid aging. Since previous studies have shown that the CASA complex regulates protein homeostasis of a cell in regards to aging, we decided to test if lamin was a direct target of the CASA complex because it is related to both aging and disease. We found that lamin levels accumulate in both p38 MAPK mutants and stv knockdown muscles. In addition, we find that lamin co-immunoprecipitates with all the members of the CASA complex. These data suggest that p38 MAPK and the CASA complex may influence aging through the turn over lamin.

34. Defining how dERR regulates growth during Drosophila Oogenesis

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Cancer cells proliferate rapidly by using an alternative metabolic system called aerobic glycolysis. Aerobic glycolysis, also known as the Warburg effect, uses carbons from carbohydrate metabolism to synthesize the proteins, lipids, and nucleotides needed for generating substantial amounts of biomass; however, the mechanism that regulates the switch to aerobic glycolysis remains poorly understood. Understanding how aerobic glycolysis and other metabolic pathways are regulated holds promise for development of new cancer treatments. Previous research has shown that the fruit fly *Drosophila melanogaster* also utilizes aerobic glycolysis to support rapid growth in larvae. Moreover, the onset of aerobic glycolysis in the fly is controlled by the *Drosophila* estrogen-related receptor (dERR), which encodes the only fly ortholog of the ERR nuclear receptor family. In order to further explore how dERR regulates biosynthesis and carbohydrate metabolism in the context of cell growth, we examined the role of this nuclear receptor in *Drosophila* oogenesis. Our analysis revealed that *dERR* is more highly expressed in stages 1 through 6 egg chambers compared to later stages of oogenesis (stages 7 through 14). Furthermore, *dERR* deficient egg chambers fail to grow past stage 5 size, suggesting that dERR is required to support cellular growth during this stage of development. Finally, we demonstrate that the dERR target gene *Lactate Dehydrogenase* (*Ldh)* is also expressed during early oogenesis in a dERR-dependent manner. These findings suggest that dERR is required for activating a metabolic program that supports oocyte development and female reproduction.

35. Growth Regulatory Pathway collaborates with Axial Patterning Genes to regulate Patterning and Growth in Drosophila Eye

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In any multicellular organism, organogenesis requires axial patterning to determine Antero-Posterior (AP), Dorso-Ventral (DV), Proximo-Distal (PD) axes. Any deviation in these axes during development leads to congenital birth defects. In our model system, *Drosophila melanogaster* (a.k.a fruit fly), Dorso-Ventral (DV) patterning marks first lineage restriction event. We have identified *defective proventriculus* (*dve*-a Homeobox gene), an ortholog of SATB homeobox 1 (special AT-rich sequence binding protein 1), as a new member of DV patterning gene hierarchy. We have shown that *dve* acts downstream of *pannier* (*pnr*, GATA-1 transcription factor), and upstream of *wingless (wg)* in dorsal gene hierarchy. Loss-of-function of *dve* or *pnr* results in dramatic dorsal eye enlargements, whereas gain-of-function suppresses the eye fate. We have demonstrated that Wg is a downstream target of Hippo growth regulatory pathway (highly conserved) in eye. Furthermore, Wingless (Wg), which acts downstream of *dve*, also exhibits similar eye enlargement and suppression phenotypes and has been shown to play a role in growth. Here, we present that DV patterning genes interact with Hippo signaling to regulate the common downstream target, Wg during growth and patterning of developing *Drosophila* eye. Our data (using Gain-Of-function and Loss- Of-function studies) states that (1) DV patterning gene pathway and hippo pathway are related and acts antagonistically of each other, (2) Activation of Hippo signaling in *dve*, *pnr* expression domain results in change of head specific cell fate to an eye by downregulating wingless (we have also tested retinal determination fate markers in these backgrounds), (3) DV

patterning genes acts downstream of hippo pathway, and that (4) DV patterning genes regulates the expression of downstream targets of hippo pathway. This study will address an important question, whether the axial patterning genes (*dve, pnr*) and Hippo pathway regulates patterning and growth independently or in-coordination with each other by regulating Wg signaling in order to form an eye/or any organ. The results from these studies will be presented.

36. HIB Ensures Hh Robustness By Preferentially Targeting CiR
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Hedgehog-induced expression of the E-3 ligase Roadkill / HIB serves to ensure Hh signaling robustness by preferentially targeting the repressor form of the transcription factor Ci. Where Hh signaling is high, Ci is prevented from being processed into the repressor form, and HIB ensures that there is no Ci repressor adjacent to the anterior-posterior boundary. Previous hypothesis holds that HIB's role is in attenuating the Full-Length form of Ci, but here we propose that HIB preferentially attenuates CiR and functions in the robustness of Hh signaling. We look at HIB's role in contributing to robustness by temperature stressing Drosophila embryos with and without the HIB gene, and we look at HIB's role in mitigating the effects of CiR by expressing CiR near the A-P boundary and expressing HIB to mitigate the effects.

37. Collective Cell Migration in the Drosophila Ovary: Connecting the Dots to Tumor Invasion
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Collective cell migration is a complex and fascinating process, fundamental not only to wound healing, immune response and embryogenesis but also to tumor invasiveness. Critical questions in this process include: how groups of cells break away from the epithelium and continue migrating as a single unit; how collectives establish dynamic cell-cell junctions to stay together; and how cells of the collective communicate with each other to coordinate their migration. The relatively simple border cells from the *Drosophila* ovary are an excellent genetic model system to study in vivo collective cell migration and invasion. The 6-10 border cells migrate collectively to the large oocyte at the posterior end of the developing egg chamber, the functional subunit of the ovary. Recently, in collaboration with the Lathia lab (Cleveland Clinic), we have found that some patient-derived glioblastoma cancer cells can undergo collective cell invasion. An RNAi screen in border cells was designed to target conserved cell-cell junction genes whose elevated expression was associated with glioblastoma patient survival. This screen revealed four top candidate genes–*α-Catenin (α-Cat)*, *dachsous*, *Lachesin* and *Symplekin*–that displayed consistent migration defects when knocked down by RNAi using a GAL4 driver expressed in both border cells and the central polar cells. Currently, we are following up on *α-Cat*, the gene with the strongest migration defect (a high percentage of partial or no migration). Further, preliminary live imaging analyses of *α-Cat* RNAi border cells showed that the border cell cluster splits along the path of migration. Using cell-specific GAL4 drivers, we found that knocking *α-Cat* down only in border cells or only in polar cells also caused the cluster to split. Future work includes analyzing mutant alleles of the top four candidate genes to confirm the phenotypes observed in the RNAi screen. We also plan to test these genes in established *Drosophila* larval tumor models to further study the roles of these genes on tumor formation and invasion.

38. Ndc80 complex members relocalize during hypoxia in female meiosis but not mitosis
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The fidelity of cell division is dependent on the spindle. In mitosis, the spindle co- orients sister chromatids via the kinetochore, and the dynamic instability of spindle microtubules allows sister chromatids to move to opposite poles of the cell. This guarantees that daughter cells contain identical

genetic material. In meiosis, this process leads to production of gametes (eggs or sperm). Kinetochore-associated proteins, one of which being the heterotetrameric ndc80 complex, facilitate the centromere-microtubule interactions, ensuring co-orientation and bipolar tension before chromosome segregation. Anomalies in this interaction delay the onset of anaphase via the activation of the spindle assembly checkpoint, inducing cell cycle arrest, and can result in aneuploidy. We have localized GFP fusions of ndc80 complex constituents, Mis12, Spc105, Nuf2, and Ndc80, in meiotic and mitotic spindles in *Drosophila melanogaster*. In female meiosis, we found that these proteins were not on meiotic prometaphase kinetochores during normoxia, but they were there during hypoxia. In contrast, by live imaging of syncitial embryos, we saw no difference in the localization of these proteins between hypoxic and normoxic mitoses. This indicates that the ndc80 complex may have a novel role in facilitating hypoxic arrest during meiosis.

39. Large Scale Genetic Screen to Identify Metabolic Regulators of Specification and Differentiation

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Metabolism is an immense and intricate network of both catabolism and anabolism. Far more than producing ATP, metabolic pathways build nucleotides, create the fatty acids that allow signaling cascades to function, and even drive the growth of certain tumors via the production of oncometabolites. Metabolism is an indispensable process, but biologists often overlook metabolism as a cellular housekeeping function. However, this view of metabolism is rapidly changing. Metabolic genes have recently been implicated in nutrient sensing, signal transduction, and the regulation of cellular differentiation. In order to further explore the links between metabolism and animal development, we are using an RNAi-based approach to identify metabolic enzymes that influence *Drosophila* eye development. We have systematically targeted 93% of all genes associated with *Drosophila* metabolism in the developing fly eye prior to cell specification using an *eyes absent* (*eya*) Gal4 driver. We then conducted a secondary screen using *GMR-Gal4*, which disrupts gene expression after eye specification, to identify those genes that are simply required for cell viability. Overall, our screen has identified ~50 metabolic genes that are required for eye development but not cell viability. The bulk of the genes implicated by the screen are involved in either the electron transport chain (ETC) or the oxidative branch of the pentose phosphate pathway. Since both pathways directly affect reactive oxygen species (ROS) levels in the cell, our screen suggests that oxidative stress plays an essential role in regulating eye specification.

40. Mito-nuclear interactions modify Drosophila exercise performance

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Endurance exercise has received increasing attention as a broadly preventative measure against age-related disease and dysfunction. Improvement of mitochondrial quality by enhancement of mitochondrial turnover is thought be among the important molecular mechanisms underpinning the benefits of exercise. Interactions between the mitochondrial and nuclear genomes are important components of the genetic basis for variation in longevity, fitness and the incidence of disease. Here, we examine the effects of replacing the mitochondrial genome (mtDNA) of several *Drosophila* strains with mtDNA from other strains, or from closely related species, on exercise performance. We find that mitochondria from flies selected for longevity increase the performance of flies from a parental strain. We also find evidence that mitochondria from other strains or species alter exercise performance, with examples of both beneficial and deleterious effects. These findings suggest that both the mitochondrial

and nuclear genomes, as well as interactions between the two, contribute significantly to exercise capacity.

41. Ribbon Regulates Gonad Development and Function

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During embryonic development, cells migrate and interact to form organs. The genes required for organ formation often function to maintain homeostasis in adult tissues as well. This is particularly true in the gonad, as many genes that regulate gonad formation, also function to maintain gametogenesis throughout adulthood. Previous studies in our lab demonstrated that the *ribbon* (*rib*) gene is required for the coalescence of germ cells and somatic gonadal cells and the compaction of the embryonic gonad. Rib belongs to the Broad Complex, Tramtrack, and Bric à brac (BTB) family of proteins that are characterized by their BTB domains, which mediate protein-protein interactions. In addition, Rib also contains a Pipsqueak DNA binding domain, and has been demonstrated to function as a transcription factor. In order to explore if Rib might also be required in later stages of gonad development, we first examined *rib* expression in adult gonads. While Rib protein localized to the nucleus of germ cells and somatic cells in the adult testis, consistent with its role as a transcription factor, it localized to the cell periphery of somatic cells in the adult ovary. To further explore the function of Rib in the adult gonad, *rib* was overexpressed in germ cells or somatic cells in males and females. While *rib* overexpression in germ cells in females had little effect, overexpression in somatic cells caused severe defects in ovary development. Overexpression of Rib in both germ cells and somatic cells in males resulted in severely truncated testis. These results suggest that too much Rib is deleterious for gonad development. *rib* was also knocked down in somatic cells and germ cells in both males and females; however, gonad defects were only observed upon knockdown of *rib* in the somatic cells of the testis, consistent with gene expression profiling that reveals stronger *rib* expression in the testis than the ovary. Given the severe defects observed upon *rib* overexpression, we have started to examine the larval stages of gonad development to understand how too much Rib leads to severely compromised gonad morphology in the adult.

42. Correlating lifespan with sleep architecture in Drosophila

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The link between health and good sleep is well known - but there is still much to learn about the biological mechanisms that govern the sleeping process and how changes in this system affects an organism's overall health. Previous efforts that examine basic sleep metrics have resulted in limited success either describing or predicting lifespan in *Drosophila melanogaster*. . But since there are increasing reports linking sleep with health, we hypothesize that sleep architecture is unique to each individual animal and that changes in these characteristics over time may be more indicative of the animal's health than basic sleep metrics alone. For example, longer sleep is not necessarily better, and it's likely that the duration of sleep needed is unique to each individual animal, with variation even within the same species.
We have been collaborating with investigators in the Department of Statistics to develop a sophisticated model that generates lifespan predictions based on fly wake and sleep transition data, along with changes in general sleep stability. We utilized *Drosophila* Activity Monitors (DAM System; Trikinetics) to track individual fly movement patterns, and built a model using sleep data from over 400 flies. Using this model, we subsequently binned flies into short-lived and long-lived cohorts after a 30 day monitoring period, allowing us to determine how biomolecular processes change with an organism's age. Using these methods, we have identified a difference in oxidative stress makers between our long- and short-lived flies.

43. Understanding the role of Wg Signaling pathway in Aβ42 mediated neurodegeneration

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Alzheimer's disease (AD), a common form of dementia and an age related progressive neurodegenerative disorder, manifests as memory loss and reduced cognitive ability. One of the hallmarks of AD is formation of the Amyloid-beta 42 (hereafter Aβ42) plaques, which triggers oxidative stress due to aberrant signaling and finally results in the death of neurons. However, the exact mechanism causing cell death is still not well understood. We misexpressed high levels of human Aβ42 protein in the developing fly retina, which mimics AD like neuropathology. In a forward genetic screen, we identified members of highly conserved Wingless (Wg) signaling pathway as modifiers of the Aβ42 mediated neurodegeneration. Misexpression of negative regulator of Wg like Shaggy kinase (sgg) or a dominant negative form of Drosophila T-cell factor ($dTCF^{DN5}$) or blocking Wg transport specifically by downregulating Porcupine (using $porcupine^{RNAi}$) rescued Aβ42 mediated neurodegeneration by reducing the number of dying cells and restoring the axonal targeting from the retina to the brain. It is also known that Wg induces cell death in the early eye developmental stage of *Drosophila.* We therefore want to understand by what mechanism and in which cells the Wg signaling is triggering cell death, whether it's the Aβ42 misexpressing cells or the neighboring wild type cells. In order to approach this question we have developed a two clone system in our lab to understand the crosstalk between the two cell populations, where we have shown that the wild type neighboring cells are undergoing cell death compared to the Aβ42 misexpressed cells.

44. Identification of the *Drosophila* Tribbles conserved COP1 binding site

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Acute myeloid leukemia, lung cancer, and cancers of the liver have low 5-year survival rates (26.9%, 18.1%, and 17.6%, respectively: NIH) and are linked to Tribbles-mediated degradation of the transcription factor C/EBPα, thus research into this mechanism could yield valuable information critical for therapeutic interventions. Tribbles proteins promote target protein ubiquitination through cooperative binding of a ubiquitin ligase at the COP1 binding site. COP1 binding sites have a well-defined amino acid sequence, present in both plants and mammals. This sequence is highly conserved among the three mammalian Tribbles homologs and is necessary for the turnover of C/EBP proteins. Interestingly, *Drosophila* Tribbles lacks a conserved COP1 binding domain while retaining the ability to direct C/EBP protein (in *Drosophila*, Slbo) turnover. In my research, we are attempting to identify the *Drosophila* Tribbles COP1 binding site and investigate its interaction with ubiquitin ligases. To-date, we have truncated a portion of the Tribbles C'-terminal tail and evaluated it in assays involving border cell migration and engrailed expression in the wing.