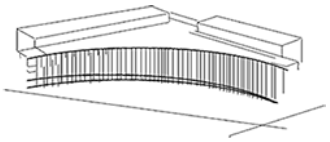
PRINCETON UNIVERSITY

Department of MOLECULAR BIOLOGY

Lewis-Sigler Institute for Integrative Genomics



Summer Undergraduate Research Program Molecular and Quantitative & Computational Biology

Poster Session

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2016

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Novel Antibacterial Lipopeptides from Marine Bacteroidetes, *Aquimarina muelleri*

Audrey Abend
Jindong Zan
Advisor: Mohamed Donia

The alarming prevalence of multidrug-resistant bacterial pathogens has made novel antimicrobial drug development a focal point for current molecular biology research. The marine environment is a rich resource of microbially secreted bioactive compounds, including lipopeptides and polyketides. Though Actinobacteria have been studied extensively as prolific producers of such compounds, our research has implicated Bacteroidetes are also rich in bioactive compounds. One such compound, a predicted lipopeptide, from the marine Bacteroidetes species, *Aquimarina muelleri*, has shown remarkable antibacterial activity in disc diffusion bioassays. Using liquid chromatography-mass spectrometry (LC-MS) analysis of organic extracts of bacterial cultures over an 8-day time course, we have shown the consistent production of this compound in comparison to previous studies, and found that cultures of Day 1 contain the highest amount of this compound. We are currently preparing 60 L of culture to isolate enough compounds for nuclear magnetic resonance (NMR) to elucidate the structure of the compound responsible for the antimicrobial activity. Furthermore, we hope to link the lipopeptide to a specific biosynthetic gene cluster (BGC) among several in the *A. muelleri* genome, predicted by a web based server called antiSMASH, by using heterologous expression and genetic manipulation. Overall, this research has promising implications for the clinical application of potent natural lipopeptides isolated from marine bacteria.

This research was made possible by the generous support of the Elkins Family Molecular Biology Senior Thesis Fund.

Modelling Growth in a Multicellular Structure

Elin Ahlstrand
Jasmin Imran Alsous
Advisor: Stanislav Y. Shvartsman

Unravelling the mechanisms underlying cell size control is a challenging and pervasive task in developmental biology. Oogenesis, or egg development in *Drosophila melanogaster*, is an attractive system to study growth in a multicellular structure. *D. mel* oocytes develop in multicellular assemblies called germline cysts, also known as egg chambers. Each wild type egg chamber consists of 16 interconnected germline cells of which one develops into the oocyte (OO) and the other 15 become nurse cells (NC), which help sustain the oocyte (hence their name). Nurse cells transfer mRNAs, metabolites, and organelles to the biosynthetically quiescent oocyte through ring canals, which result from four synchronous divisions of a founder cystoblast with incomplete cytokinesis. While initially all 16 cells are of roughly equal size, throughout their development, the oocyte visibly grows disproportionately compared to the other cells. Furthermore, as the egg chamber adopts a more ellipsoidal shape, the nurse cells begin to show a polarized distribution of sizes, which only becomes more pronounced throughout oogenesis, with nurse cells most adjacent to the oocyte having nearly double the volume of those furthest away. Thus far, no computational models exists to explain the observed pattern of cell growth in this interconnected multicellular structure. We created a toy model with several parameters to simulate cystocyte growth in these multicellular egg chambers.

This research was made possible by the generous support of the Lewis-Sigler Institute for Integrative Genomics.

Cross-Species Comparison of Nuclear-Cytoplasmic Ratio at the Midblastula Transition in the *Drosophila* Genus

Katherine Angier
Karen Zhang
Advisor: Amanda Amodeo

The complexity of mammalian embryonic development provides a daunting challenge for science. Toward this end, the study of simpler animals is useful for building a solid foundation in our understanding of embryogenesis. Many mysteries remain even in the well-studied model organism, *Drosophila melanogaster.* In *D. melanogaster* development, major changes in gene expression and cell behavior occur at a stage known as the midblastula transition (MBT). However, the timing mechanisms that coordinate these numerous changes, such as cellularization of the syncytial blastoderm and the onset of zygotic transcription, are unknown. One metric, the nuclear-cytoplasmic ratio (NCR), has been shown to influence the timing of some, though not all, of these changes. We measured the NCR in different species of *Drosophila* with varying relatedness to *D. melanogaster* to determine whether the metric scales as embryo size and genome length changes. We found all species appeared to undergo the same number of divisions, entering the MBT at cycle 14. Without accounting for genome length, the relationship between NCR and volume is approximately hyperbolic. Accounting for genome size, the sequenced species displayed significant differences in NCR when normalized to the NCR of *D. melanogaster*. This suggests that the embryo's ability to measure its MBT may vary between species. We aim to examine more species in search of correlations between the NCR and metrics such as embryo volume, time to MBT, and phylogenetic relatedness. Better characterization of the NCR across Drosophila species may lead to clues on how the embryo detects and uses this ratio to coordinate aspects of the MBT.

This research was made possible by the generous support of the Lewis-Sigler Institute for Integrative Genomics.

Investigating the Genetic Basis of Extracellular Electron Transfer in *Shewanella oneidensis* by Knockout Sudoku

Isao Anzai
Oluwakemi Adesina, Liat Kugelmass
Advisor: Buz Barstow

As global demand for energy increases, there is pressure to adopt cleaner and more efficient energy sources than fossil fuels. Biological photosynthesis provides a template for harnessing the tremendous solar energy flux, but while photosynthesis is quite efficient with respect to downstream metabolism occurring intracellularly, the initial light-harvesting step is not. Instead, photovoltaic energy capture is far more efficient, proposing a solution that combines photovoltaic capture efficiency with the flexibility of microbial metabolism. Nature provides specialized electrosynthetic microbes, such as the bacterium *Shewanella oneidensis*, with a machinery known as extracellular electron transfer (EET) to connect renewable electricity with metabolism; however, a full genetic understanding of EET is lacking. Thus, we have developed Knockout Sudoku, a novel method for producing gene knockout collections, to investigate unknown EET gene functions. We have used the method to create a collection for *S. oneidensis* and have discovered genes previously not known to be involved in EET. My thesis will investigate the genetic basis of electron uptake in *S. oneidensis* using a reduced colorimetric dye that turns clear upon oxidation. By testing a variety of electron acceptors such as nitrate and fumarate and carefully analyzing the mutants defective in oxidation, we can begin to paint a clearer picture of how EET is occurring. This research will guide our ability to engineer microbes with more efficient electron transfer systems with the aim of creating better technologies for renewable energy storage.

This research was made possible by the generous support of the Susan W. and James C. Blair '61 P87 Endowed Senior Thesis Fund.

The Role of Cholinergic Neurons in the Nucleus Basalis and Medial Septum in Working Memory

Jennifer Au
Advisor: Ilana Witten

Working memory is the basis of fundamental learning and cognition, as it involves the updating, maintenance, and manipulation of novel information to direct subsequent behavior. Working memory has been linked to the cholinergic system through pharmacological interventions, but effective mediation of working memory disorders, such as Alzheimer's disease, has been unsuccessful. This may be due to the slow timescale of action of pharmacology, suggesting a fast temporal component of cholinergic activity. Optogenetics may be a promising solution to this difficulty, as it directly mediates neuronal activity at a rapid timescale that matches cholinergic neuronal activity. To investigate the role of cholinergic neuronal activity in working memory, cholinergic neurons in the nucleus basalis (NB) and medial septum (MS) can be selectively inhibited using optogenetics during a working memory task. Preliminary results show a divergence in NB and MS activity, in that activity in the NB is higher during incorrectly performed trials of a working memory task, whereas MS activity is higher during correctly performed trials. This observational data suggests different functions of cholinergic neurons in the NB and MS, and is the motivation for direct inhibition of cholinergic neurons using optogenetics.

This research was made possible by the generous support of the Crecca '46 Molecular Biology Senior Thesis Fund.

dNTP metabolism in early *Drosophila melanogaster* embryos

Debopriyo Biswas
Advisor: Stanislav Y. Shvartsman

Ribonucleotide reductase (RNR) is responsible for all *de novo* synthesis of deoxyribonucleoside triphosphates (dNTPs) in cells, as it catalyzes the reduction of nucleoside diphosphates (NDPs) to dNTPs. A precise balance of dNTPs is required for DNA replication and repair accuracy. A complex scheme of allosteric regulation of RNR by dNTPs ensures balanced dNTP concentrations. Misregulation of dNTP concentration caused by mutations in RNR leads to high mutation rates which can cause genomic instability and disease. dATP and ATP compete for the activity site on RNR, where dATP inactivates the enzyme and ATP activates RNR. dATP, dTTP, ATP and dGTP bind to the specificity site and determines which NDP molecule is reduced at the catalytic site. Principles of RNR allosteric regulation have been well established only in *in vitro* experiments.

We used *Drosophila melanogaster* embryos as a system to investigate RNR activity in multicellular organisms *in vivo*. In the first 2 hours after fertilization, the embryo undergoes 13 rapid synchronous nuclear divisions, by the end of which the embryo has around 6000 nuclei. For these divisions to happen, the embryo needs abundant supply of dNTPs for DNA replication. When we stopped RNR activity by injecting hydroxyurea, a small molecule inhibitor of RNR, embryos failed at the 11th division. Co-injection of dNTP's rescued the death induced by HU. This showed that RNR was highly active, and supplied for most of the embryo's dNTP demand during nuclear division cycles by RNR activity. Therefore, we were able to confirm the allosteric regulatory principles established *in vitro* by injecting dNTP molecules into the developing embryo and observing cell cycle success rate.

This research was made possible by the generous support of the Lewis-Sigler Institute for Integrative Genomics.

Long-range spatial correlations and positional information in the early fly embryo

Helena Casademunt
Advisors: William Bialek and Thomas Gregor

During early embryonic development of the fruit fly *Drosophila melanogaster*, the position of each cell is determined through the expression levels of a set of genes that form a complex network. Recent simultaneous imaging of the expression levels of four gap genes and three pair-rule genes generated data that allows us to examine interactions between genes and information flow in the network. Previous work has shown that the expression levels of the gap genes contain enough information to specify the position of each cell along the anterior-posterior axis of the embryo with a precision of about 1% of the embryo's length. We have observed that the stripes that characterize the expression pattern of the pair-rule genes are positioned with the same precision. However, through spatial correlation analysis, we reveal that the relative positions of these stripes show less variability than their absolute positions, which puts constraints on the true information content in the system. Continuation of this work aims at analyzing the spatial correlations that exist in the network at the levels of both the gap and pair-rule genes. First, the correlations between the position of features in the genes (peaks, troughs or boundaries of expression levels); second, the correlations between gene expression levels at different positions. This will be a step towards elucidating the mechanisms through which the fate of each cell is determined and the flow of information that governs these mechanisms, which are essential for the development of the embryo and the foundation for the later development of the fly.

This research was made possible by the generous support of the Lewis-Sigler Institute for Integrative Genomics.

Real-Time Segmentation of Fruit Fly Courtship Songs Through a Deep Convolutional Neural Network

Minseung Choi
Adam J Calhoun
Advisor: Mala Murthy

During the courtship of *Drosophila melanogaster*, the male fly produces dynamically-patterned songs with its wings while chasing the female until mating occurs. These songs consist of two primary modes -- sine and pulse -- which are interspersed with periods of no song. Although an offline algorithm has been recently developed for the segmentation of song recordings into the three modes, the emergence of optogenetic approaches, which permit real-time activation of neurons, calls for an online algorithm. Here, we train a deep convolutional neural network (CNN) for real-time classification of song recordings, with a training set developed from the outputs of the existing offline algorithm. Existing knowledge about song structures were utilized in designing the CNN architecture. We can detect changes in song modes and respond optogenetically within at most 40 milliseconds, with a classification rate of 94.8%. The online algorithm developed here, in conjunction with existing behavior-recording chambers, now enables real-time manipulation of courtship behavior in a closed-loop manner within the short timescale of a song mode.

This research was made possible by the generous support of the Lewis-Sigler Institute for Integrative Genomics.

The Role of the US9/PACS-1 Interaction in Pseudorabies Virus Anterograde Spread

Rachel Choi
Orkide Koyuncu, Julian Scherer
Advisor: Lynn Enquist

Pseudorabies virus (PRV) is a pathogen that resides in the nervous system of the host animal, localizing to the peripheral nervous system but also spreading to the central nervous system on rare occasions. Although it does not infect humans, PRV may cause pruritus and Aujeszky's disease in agricultural animals like cattle, sheep, and pigs. In addition, it is closely related to the human pathogen herpes simplex virus (HSV). Therefore, it is important to understand how PRV causes disease through molecular mechanisms.

This study focuses on understanding how PRV utilizes viral and neural components to spread throughout the host. In previous studies, US9, a type II viral membrane protein, has been identified as critical for PRV axonal sorting and transport from the cell body towards the ends of the axons (anterograde transport). We were interested in looking at potential interactions of US9 with host cell proteins. A preliminary experiment in the lab suggested that US9 may interact with phosphofurin acidic cluster sorting protein 1 (PACS-1), a host cytosolic protein. Therefore, we were interested in two aims: further investigating the interactions between US9 and PACS-1 on a biochemical level, and determining the role of PACS-1 in PRV transport and spread. We designed and developed shRNA constructs to target and knock down PACS-1 in neurons, so that we could assess the role of PACS-1 in PRV axonal sorting. Ultimately, by studying the role of PACS-1 in PRV spread, we hope to contribute to research on non-classical drugs that target PRV and related viruses at the level of viral spread.

This research was made possible by the generous support of the Crecca '46 Molecular Biology Senior Thesis Fund.

Examining Lasso Peptide Uptake by TonB Dependent Transporters

Aastha Chokshi
Joe Koos
Advisor: A. James Link

Lasso peptides are a class of ribosomally synthesized and post translationally modified peptides (RiPPs), and are attractive candidates for engineered protein drugs as they are resistant to proteases. Astexin-2 and Astexin-3 are lasso peptides synthesized by *Asticcacaulis excentricus*. However, their uptake into bacterial cells is relatively unknown. There are two lasso peptide gene clusters in *A. excentricus*. The gene cluster codes for proteins responsible for the synthesis and maturation of lasso peptides as well as other proteins hypothesized to be involved in the transport of lasso peptides. The AtxF gene is homologous to genes encoding other Ton-B dependent transporters (TBDTs), which transport metal ions bound to siderophores. TBDTs have been shown to interact with sigma regulator and sigma factor proteins, which are homologous to FecR-like and FecI-like proteins in the *A. excentricus* gene cluster. As a result, it has been hypothesized that AtxF interacts with FecR and FecI to transport lasso peptides within the bacterial cell. This project will test whether heterologously expressed AtxF, FecR and FecI in *Escherichia coli* can successfully transport lasso peptides. Investigating the mechanism of lasso peptide uptake into bacterial cells can also provide insight into their function.

This research was made possible by the generous support of the Elkins Family Molecular Biology Senior Thesis Fund.

A morphogenetic role for FGF signaling in zebrafish cardiac looping and ballooning

Briana Christophers
Meagan Grant
Advisor: Rebecca D. Burdine

The cellular events underlying proper cardiac development require the correct interpretation of signaling cues. Congenital heart defects, which affect 1 in 100 infants in the U.S. each year, arise from a failure of these events to occur. Using zebrafish as a model, we aim to better understand the links between errors in signaling, cellular aberrations, and heart malformation. To that end, we have investigated the role of Fibroblast Growth Factor (FGF) signaling, which is known to couple morphogenesis to cell migration events in zebrafish, in asymmetric heart development. We have uncovered a role for FGF signaling in the later stages of heart development that is independent of its earlier role in establishing left-right asymmetry. We find that FGF signaling is critical for proper chamber placement (cardiac looping) and chamber expansion (cardiac ballooning), but during different developmental windows. We hypothesize that FGF signaling influences cardiac looping by promoting the addition of a late-differentiating pool of cardiac progenitors (second heart field) to the arterial pole of the heart tube, and that the pathway influences cardiac ballooning by regulating cell shape changes in the curvatures of the looped heart. Future experiments will make use of transgenesis, time-lapse microscopy, and photocoversion to test these hypotheses.

This research was made possible by the generous support of the Genentech Foundation.

Effect of Extending Stimulus Duration on Rats for Accumulation of Evidence Task

Dominick Cioppa
Advisor: Carlos Brody

Drift diffusion models for accumulation of evidence tasks have been able to accurately fit behavioral patterns in rats and humans. Previous work from our laboratory developed a detailed method for quantitative modeling of the decision making process within an auditory task known as the “Poisson clicks task.” This model allows rat behavior to be fully captured. While the model found that rats accumulate sensory evidence equally over the entire duration of the sensory stimulus, the subjects’ behavior has not been tested beyond one second of stimulus length. All accumulation of evidence tasks, including the Poisson Clicks task, have an underlying working memory component, and it is possible that evidence accumulation could degenerate with the greater demand of lengthened stimuli. We will investigate this by first training rats in a version of the task using stimuli of much longer duration, and will quantify behavior using psychophysical reverse correlations and psychometric plots. We will then reversibly inactivate with muscimol the posterior parietal cortex (PPC), widely known to influence working memory, to probe for a role of the PPC involvement in the lengthened stimulus task. Although previous inactivation of the rat PPC showed no effect on the short-stimulus Poisson clicks task, an auditory delayed comparison task with long working memory duration was found to be impaired after PPC inactivation, suggesting that the PPC may be necessary for our long-stimulus-duration version of the Poisson Clicks task. Furthermore, the extent of projections to the PPC from the retrosplenial cortex, an area thought to be relevant for long timescale integrations, will be probed using retro/antero-grade tracing techniques. This projection mapping will gather information relevant to the possibility of inactivating this portion of the brain for our modified Poisson Clicks trials, especially if the PPC is shown to have an effect on accumulation.

This research was made possible by the generous support of the Elkins Family Molecular Biology Senior Thesis Fund.

Regulation of *dand5* via Flow sensation and miRNAs

Marvin Cortez
Jose Pelliccia
Advisor: Rebecca Burdine

Left-right (LR) development of internal organs is highly conserved among vertebrates. Deregulation of factors controlling this process contributes to developmental defects, such as those leading to congenital heart defects. LR patterning in zebrafish is regulated early in development by leftward fluid flow generated by cilia of cells lining Kupffer's Vesicle (KV). This is proposed to lead to increased intracellular Ca^{2+} concentrations which eventually down-regulate the nodal inhibitor, *dand5*, on the left of KV. A cation channel in the cilium, Pkd2, is believed to be responsible for transporting Ca^{2+} into cells. This down-regulation of *dand5* in KV, however, is lost in *pkd2* mutants. Recent studies argue against this mechanosensory mechanism and support the hypothesis that Pkd2 localized to the endoplasmic reticulum (ER) is responsible for increases in Ca^{2+}. *pkd2* mutant zebrafish exhibiting curly tails and heart jogging defects were partially rescued by a *PKD2* variant capable of localizing to the ER. It remains to be determined if Pkd2 is localized to cilia in these rescued embryos. We were additionally interested in identifying the effector responsible for *dand5* regulation further downstream of flow sensing. Using morpholino target protectors (TP), we showed that *dand5* in KV is downregulated by miRNAs. Embryos injected with *dand5*-TP exhibited randomized heart laterality scored by left (wildtype), right, or centered jogging. Further investigations are needed to determine the connection between flow sensation, Pkd2 activity, and asymmetric expression of *dand5* in KV.

This research was made possible by the generous support of the Department of Molecular Biology and the Lewis-Sigler Institute Program for Diversity and Graduate Recruitment.

Investigating the *rhl* quorum-sensing system in *Pseudomonas aeruginosa* biofilms

Michael Delgado
Sampriti Mukherjee
Advisor: Bonnie L. Bassler

Pseudomonas aeruginosa is a potent human pathogen that infects host tissues by forming multicellular communities called biofilms. Quorum sensing (QS), a mechanism of bacterial cell-cell communication, is necessary for virulence and biofilm formation in *P. aeruginosa*. The *P. aeruginosa* QS circuit consists of two autoinducer synthase/receptor pairs, LasI/R and RhlI/R, which produce and detect homoserine lactones, 3OC12-HSL and C4-HSL, respectively. At high cell density, LasR and RhlR bind their cognate autoinducers, dimerize, bind DNA, and activate gene expression required for virulence and biofilm formation. While wild-type (WT) *P. aeruginosa* form biofilms with wrinkled centers and smooth peripheries on Congo red semi-solid agar, we find that Δ*rhlI* and Δ*rhlR* mutants form smooth and hyperwrinkled biofilms respectively. We would expect Δ*rhlI* and Δ*rhlR* mutants to phenocopy as is the case in Δ*lasI* and Δ*lasR* strains. I hypothesize that RhlR binds to another ligand in the absence of C4-HSL and activates transcription of a subset of genes in the RhlR regulon. To identify genes involved in the alternative pathway, I am performing Tn5 insertion mutagenesis in the Δ*rhlI* strain and screening for mutants that confer a 3-fold decrease to Δ*rhlR* levels of expression of *rhlA*, a RhlR-dependent QS gene. In parallel, I am probing RhlR with small epitope tags for biochemical assays and structural studies. While $RhlR^{\Delta 18\text{-}26::HA}$ and $RhlR^{\Delta 18\text{-}25::FLAG}$ proteins do not produce the RhlI-dependent virulence factor pyocyanin, they drive *rhlA* expression to nearly WT levels. Perhaps insertion of these tags between amino acids 18-26 locks RhlR in a conformation that binds the alternate ligand with higher affinity. To this end, I plan to pull-down RhlR bound to its alternate ligand with anti-FLAG antibody for LC-MS and NMR to elucidate the structure of the alternate ligand.

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Disrupting MHCI cytoplasmic domain's interactions in the brain: a peptide-based approach to stroke recovery

Peter Du
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Mice lacking the classical major histocompatibility complex class I (MHCI) genes H2-K^b and H2-D^b show improved recovery from experimentally-induced ischemic brain injury, suggesting MHCI-deficiency is associated with neuroprotection. However, the molecular mechanisms by which loss of MHCI enhances stroke recovery remain unknown, and it is unknown if similar protection can be extended to a genetically normal organism. This study aims to acutely block neuronal MHCI function *in vivo,* with the goal of promoting stroke recovery in wildtype mice. Preliminary results suggest that MHCI normally inhibits neuroprotective synaptic *N*-methyl-D-aspartate receptors (NMDARs) via interactions with the PDZ domain of synaptic protein spinophilin. Previous work shows that the cytoplasmic domain (CD) of MHCI, when delivered into single neurons as peptides, can act as decoys and block endogenous MHCI's inhibitory effects, thus promoting synaptic NMDARs responses. In this study, we began to extend these findings to an *in vivo* setting. To deliver the peptide into cells, the $MHCI_{CD}$ was fused to Tat, an HIV-derived cell-penetrating peptide (CPP). The fusion peptide was cloned into a vector, purified, and confirmed to bind *in vitro* to PDZ ligands. A tyrosine mutant version of the peptide was also created and showed decreased binding to PDZ ligands. The fusion peptide was confirmed to have the ability to be taken up by cultured hippocampal neurons. These results pave the way for expansion of the use of the Tat H2-K_{CD} peptide *in vivo* and for electrophysiology and synaptic scaling studies. This study could ultimately help identify a novel approach to improve stoke recovery and provide a novel tool to study MHCI's function *in vivo* with improved temporal resolution.

This research was made possible by the generous support of the Evnin '62 Molecular Biology Senior Thesis Fund.

Augmin Complex Assembly

Elbegduuren Erdenee
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Advisor: Sabine Petry

Microtubules (MTs) are cellular structures involved in many eukaryotic cell processes, including forming the mitotic spindle for chromosome segregation. The mitotic spindle assembly is dependent on a branching MT nucleation pathway, in which new MTs grow from the sides of existing MTs. One of the protein complexes essential to branching MT nucleation is the augmin complex, depletion of which leads to aberrations in spindle bipolarity such as a lack of axial symmetry and spindle elongation as well as to the absence of MT branching events. Although it is known that augmin plays an essential role in branching MT nucleation, interactions between its eight subunits (H1-H8) and structure are not known, thus a precise mechanistic role for augmin has yet to be established. In this project, we performed dimer identification screens using a pulldown assay in which augmin subunits were systematically co-expressed in insect cells and purified to check for interactions. We then tested sub-complex formation from promising interacting subunits using size exclusion chromatography. Here, we show the formation of a stable dimer (H1-H4) and a tetramer (H1-H4-H3-H5) as well as a pattern of possible interacting partners, but further confirmation of these interactions is needed. We are in the process of testing more subcomplex formation and analyzing the MT binding properties of tested sub-complexes and plan to proceed with crystallization trials for the most promising sub-complex. Knowing augmin assembly and ultimately its crystal structure will provide valuable insight into augmin's function in branching MT nucleation, which is a major mechanism for amplifying MT density in the spindle, maintaining its local structure and polarity given rapid MT turnover.

This research was made possible by the generous support of the Elkins Family Molecular Biology Senior Thesis Fund

Gene regulation via interallelic interactions during *Drosophila* development

Tyler Fair
Bomyi Lim
Advisor: Michael S. Levine

Transcription of developmental patterning genes in *Drosophila melanogaster* is finely regulated to ensure precise spatiotemporal expression across coordinately developing cells. Fine-tuning is achieved through the combined actions of several recently identified regulatory mechanisms, including transcriptional memory, shadow enhancers, 'poised' enhancers and promoters, and intra-enhancer redundancy. However, it remains unknown whether interactions between homologous alleles control gene expression during development. Using RNA live-imaging methods and quantitative analysis, I examine and compare the transcriptional dynamics of living homozygous and heterozygous *Drosophila* embryos containing reporter genes with MS2 and PP7 stem loops under the control of well-defined enhancers of patterning genes including *brinker*, *snail*, and *Krüppel*. By tagging maternal and paternal alleles with distinct fluorophores, I explore the potential for synchronous biallelic expression, allele by allele silencing, and a sex-specific bias in transcriptional output. I also investigate transheterozygotes where one of the enhancers is full-length and full-strength, while the other is minimal and weak, to observe *trans* interactions between homologs. These experiments aim to elucidate a novel mechanism of transcriptional regulation during development.

This research was made possible by the generous support of the Evnin '62 Molecular Biology Senior Thesis Fund.

Investigating the Role that the G3BP protein Rasputin Plays in regulating *nanos* expression during early *Drosophila* development

Benjamin A. Gallo
Joel V. Tamayo
Advisor: Elizabeth R. Gavis

The regulation of gene expression after a transcript has been produced is mediated by a multiplicity of processes that target the mRNA or protein. Disruptions of these modes of regulation have been implicated in numerous diseases, but the molecular mechanisms involved are not fully understood. During early *Drosophila* development, the regulation of the maternally-provided *nanos* (*nos*) mRNA in the developing oocyte serves as a model to study gene regulation downstream of transcript synthesis. Recently, a potential interaction has been identified between the *nos* 3'untranslated region (*nos 3'UTR*) and Rasputin (Rin), the only *Drosophila* homologue of G3BP, a multi-functional protein conserved across eukaryotes that is overexpressed in numerous human cancers and involved in various biological processes. Although preliminary results suggest that Rin is a positive regulator of *nos* expression, it is unknown whether Rin targets *nos* mRNA or Nos protein. We aim to determine whether Rin regulates *nos* post-transcriptionally or post-translationally and to describe the molecular interactions that explain how Rin regulates *nos.* By analyzing ovaries from flies expressing an *egfp-nos-tub-3'UTR* transgene in *rin* mutants through immunoblotting, we have confirmed that Rin is required for the expression of endogenous Nos and we have found that Rin does not regulate *nos* by interactions with the *nos 3'UTR*. Further characterization of Rin-mediated *nos* regulation will contribute to our understanding of the multi-functionality of G3BP proteins and inform us about ways that this family of proteins can participate in gene regulation.

This research was made possible by the generous support of the Elkins Family Molecular Biology Senior Thesis Fund.

Examining *P. aeruginosa* Growth and Virulence on Different Stiffnesses of Polyacrylamide Gel

Ruby Guo
Sara Chuang
Advisor: Zemer Gitai

Pseudomonas aeruginosa is a common opportunistic pathogen that exists in the soil, water, atmosphere, and in the human body. However, it is also a major contributor to antibiotic-resistant hospital-acquired infections, targeting immunocompromised patients or those who have chronic or acute conditions such as cystic fibrosis or burn wounds. Unlike other pathogens, which generally infect one system in the body, Pseudomonas is broadly virulent, infecting lung, skin, sinuses, and even the eyes. We sought to understand how Pseudomonas reacts to tissues of different Young's moduli by examining their behavior on polyacrylamide gels ranging from 0.75kPa to 5 kPa, values confirmed with parallel-plate rheology. RNAseq analysis will reveal the transcriptional response of the bacteria to these various surfaces. Using single-cell imaging, it was found that there was no difference in the growth rate between different stiffnesses, which means that there will be a small growth response seen in the RNAseq results. While the RNAseq data is searching for surface regulators, an important output for surface detection is whether or not virulence is activated. Using a host killing assay, we are able to quantify the virulence of bacteria in different conditions. Death of the model organism, Dictyostelium discoideum, allow for quantification of virulence. These experiments help us better understand Pseudomonas's surface-sensing mechanism and subsequent broad pathogenicity, knowledge which is helpful in preventing the frequently fatal infections prevalent in hospitals today.

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Variance in Aggregation in *D. Discoidium*

Gavin Hall
Allyson Sgro
Advisor: Thomas Gregor

When starved, many individual *Dictyostelium discoidium* cells aggregate into fruiting bodies, where the cells in the upper part of the fruiting body ("the spore") have a further chance at survival by latching onto passing objects and the cells in the lower part of the fruiting body ("the stalk") die and structurally support the upper cells. Thus there is a clear fitness advantage to a cell ending up in the spore rather than the stalk when aggregation occurs.

Previous research has indicated that for chimeric mixtures of cells the different strains are represented in the spore and stalk at differential rates. From a fitness perspective, this means that when chimeric fruiting bodies form, one strain will consistently "win" over the other.

This research aims to correlate this fitness differential to the particular dynamics of *Dictyostelium* signaling. In this work we use wavelet analysis to connect experimental footage of cAMP waves in *Dictyostelium* populations to particular features of a model of excitable oscillatory dynamics. From this, we can isolate the essential features of the signaling patterns of each strain and correlate known fitness results with particular features of the signaling network.

This research was made possible by the generous support of the Lewis-Sigler Institute for Integrative Genomics.

Role of C-Abl kinase in protein scavenging of pancreatic cancer cells

Aiden Seunghun Han
Michel Nofal
Advisor: Joshua D. Rabinowitz

Pancreatic ductal adenocarcinoma (PDAC) is one of the leading causes of cancer deaths in the United States with extremely poor prognosis. More than 95% of PDAC is characterized by mutations in *KRAS*-oncogene, and recent studies have shown that PDAC cells depend on the process of protein scavenging for the uptake of nutrients. In this process, serum proteins are endocytosed by macropinocytosis and degraded into monomeric amino acids in the lysosome. This mode of nutrient uptake has emerged as a way of ensuring survival for PDAC cells in nutrient-poor conditions, but detailed understanding of genes and pathways involved is lacking. In this study, a genome-wide CRISPR-Cas9 screen in murine PDAC cells identified *VASP* and *Wasf2*, the genes encoding actin regulatory proteins, as two of the essential genes regulating protein scavenging. Interestingly, both are shown to be regulated by c-Abl kinase. We found that blocking c-Abl activity by nilotinib, a tyrosine kinase inhibitor, impairs proliferation of PDAC cells in leucine free medium. Flux measurements using a flux analysis method developed in our lab also showed reduced protein scavenging with nilotinib treatment. In addition, a western blot of Crkl and phospho-Crkl indicated increased c-Abl activity in amino acid depleted conditions. Altogether, these data suggest that c-Abl activity upregulates protein scavenging of PDAC cells. To further support this idea, c-Abl will be overexpressed in different cell lines to test the sufficiency of c-Abl activity for activation of protein scavenging. Completion of this study will expand our understanding of the way starved pancreatic cancer cells gain nutrients and could potentially provide a novel approach to develop drugs for pancreatic cancer.

This research was made possible by the generous support of the Elkins Family Molecular Biology Senior Thesis Fund.

Quantitatively measuring mtDNA in *Drosophila* after exposure to free radicals and antioxidants

Matthew Hankins
Advisor: Julien F. Ayroles

Free radical theory has provided a theoretical framework for researchers to investigate cellular damage due to reactive oxygen species (ROS). The theory proposes that mitochondrial DNA (mtDNA) is susceptible to damage from ROS and could be a driving force in age related illnesses. ROS are produced during aerobic respiration and it is the job of the mitochondria to protect the cell from these reactive molecules. The mtDNA is thus prone to ROS damage. We used *Drosophila melanogaster* because there are many tools available to study the mtDNA of this organism. An inbred line of *D. melanogaster* was exposed to either the free radical menadione sodium bisulfite (MSB), or to the antioxidant ascorbic acid. After treatment, the mtDNA copy number was measured using quantitative PCR. We expect the flies exposed to MSB to have a lower mtDNA copy number relative to the control while the flies exposed to the ascorbic acid to have a slight increase in mtDNA. These results would imply that mtDNA is affected by ROS, but could also be protected by antioxidants.

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Using C. elegans to Understand the Function of Branched Chain Amino Acids in Parkinson's Disease

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Advisor: Coleen T Murphy

Parkinson's disease (PD) is an age-related neurodegenerative disease that causes death or impairment of dopaminergic neurons located in the substantia nigra of the human brain. Few genes have been linked to PD, and the molecular mechanisms of this disease are largely unknown . Moreover, identifying and testing the role of new candidate genes in mammalian models of PD is slow and low throughput. To identify and rapidly test new PD genes, C. elegans gene networks and movement assays were used to model PD. Network analysis was performed using the C. elegans orthologs of candidate human PD-associated genes identified from GWAS studies . The top candidates were knocked down in C. elegans adults, then tested for thrashing defects at day 2, 5, and 8 of adulthood to identify age-specific defects. Knockdown of many of the genes, particularly bcat-1, showed defects. bcat-1 encodes Branched Chain Amino Acid Transaminase-1, which is required for the oxidation of branched chain amino acids (BCAAs). Defects in human BCAA metabolism can lead to several metabolic disorders. Interestingly, a previous report showed that C. elegans exposed to bcat-1 RNAi or increased BCAA levels have an increased lifespan and maximum velocity (MV), in contrast to the defects we find in our age-related thrashing assay. Our first goal is to test whether bcat-1 RNAi increases MV (a health span metric) when knocked down in whole animals or specifically in neurons. We performed MV assays with C. elegans exposed to bcat-1 RNAi or vector control with age. We are also testing whether the loss of BCAT-1 enzymatic activity or instead the accumulation of BCAAs is the cause of the PD-related behavior. This work will clarify the role of bcat-1 and BCAA metabolism in lifespan and PD-related health span to determine if BCAA treatment is beneficial or harmful to humans.

This research was made possible by the generous support of the Department of Molecular Biology and the Lewis-Sigler Institute Program for Diversity and Graduate Recruitment.

Characterization of Mechanisms Underlying Hepatitis B Viral Persistence

Tiffany Huang
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Advisor: Alexander Ploss

Approximately 400 million individuals worldwide are chronically infected with Hepatitis B virus (HBV). These individuals can develop fibrosis, cirrhosis, and liver disease. Current antiviral treatments for HBV are effective in reducing the viral load, but they are rarely capable of curing the disease. HBV has a limited tropism, only infecting human hepatocytes, and is able to persist through the formation of covalently closed circular DNA (cccDNA), which is an extrachromosomal viral episome that serves as the template for transcription of all viral mRNAs. To develop a drug testing platform, a reliable *in vitro* system must be established that is capable of supporting persistent HBV infection. Commonly used hepatoma cell lines do not adequately recapitulate the complex physiology of adult hepatocytes, likely confounding and complicating *in vitro* studies of HBV. Though cultures of primary human hepatocytes (PHH) provide a physiologically and clinically relevant system to study HBV infection, it is challenging to maintain hepatocyte function *in vitro*. However, new approaches including random co-cultures (RCCs) of PHHs and non-parenchymal cells have shown maintenance of functional apical and basal polarity, high levels of cytochrome P450 activity, and gene expression profiles on par with freshly isolated hepatocytes. Using these RCC-PHHs, we have established persistent HBV infection and demonstrated this system's ability to be used as a drug screening platform after treatment of PHHs with entecavir and IFN-2A. Furthermore, we have generated CRISPR/Cas9 constructs for gene knockouts of *Smc5/6* and *DDB1* to elucidate the roles of the HBx protein in the HBV life cycle. In addition, the generation of a new CRISPR/Cas9-based cccDNA reporter system will enable the sensitive detection of cccDNA.

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Where do early *Drosophila melanogaster* embryos get dNTPs?

Ariane Imulinde Sugi
Advisor: Stanislav Y. Shvartsman

During the first two hours of development, *Drosophila melanogaster* embryos undergo 13 rapid and synchronous nuclear divisions. A balanced supply of different deoxyribonucleoside triphosphates (dNTPs) is required for an active DNA synthesis prior to each cell division. As a consequence of the growth in the number of nuclei, there is an exponential increase in the demand of dNTPs throughout early development. If the embryo was to store all the dNTPs it needs for all 13 nuclear cycles, the total concentration would be 200µM of each dNTP. Imbalanced concentration of dNTPs in cells is known to be highly mutagenic. Thus, while the embryo stores some of the metabolites before fertilization, dNTPs are produced as needed during the cell division cycles. The enzyme ribonucleotide reductase (RNR) plays a major role in cell division cycle by synthesizing all the four dNTP monomers. RNR enzyme converts ribonucleotides to deoxyribonucleotides and its activity is strictly regulated allosterically. We used LC-MS to measure the concentration of dNTP in *D. melanogaster* embryo at different stages of embryonic development and before fertilization. The results showed that the mechanism producing dNTPs is activated at fertilization. In addition, the concentration measured was clearly 10 fold lower than the amount of dNTPs necessary for all the 13 nuclear divisions. Altogether, we show that *D. melanogaster* embryos need to synthesize ~ 90% of the dNTP consumed during rapid cell cycles.

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Effects of Advanced Glycation Endproducts on Focal Adhesions in Tumor Cells

Obi Iregbu
Maria Vega, Alexandra Pastino
Advisor: Jean E. Schwarzbauer

Diabetic kidney disease is characterized by damage to the blood-filtering capillaries of the kidneys due to increased deposition of extracellular matrix (ECM). This ECM dysregulation correlates with increased levels of advanced glycation end-products (AGE) on endogenous proteins. Extracellular AGEs can bind to their transmembrane receptor RAGE to mediate signaling, and increased AGE modifications on proteins correlate with both high glucose levels and increased activation of transmembrane integrin receptors. The extracellular domain of integrin anchors and activates fibronectin (FN), which forms a matrix that binds other core ECM proteins to allow for their deposition. The integrin intracellular domain connects to the actin cytoskeleton through accessory proteins such as talin, vinculin, and paxillin. The resulting attachment from the cell to the ECM, called a focal adhesion, allows for mechanical and chemical signaling across the connection. I hypothesize that AGEs binding to RAGE will alter the expression of focal adhesion accessory proteins and kinases, and that these effects are mediated through a p38-regulated/activated kinase (PRAK/MK5)-dependent pathway. Immunofluorescence for vinculin has has shown an increase in its expression after AGE-BSA treatment. In my future experiments, I will verify these changes by measuring mRNA and protein levels of vinculin, talin, and paxillin and the activation of focal adhesion kinase, integrin-linked kinase, and PRAK with and without AGE-BSA treatment. Because PRAK works downstream of RAGE, I will also knockdown PRAK with siRNA and investigate the effects of its knockdown on focal adhesion organization, integrin activation, and FN matrix assembly. Findings that AGEs acting through RAGE to increase the activity and expression of focal adhesion accessory proteins and kinases will provide evidence linking downstream RAGE signaling pathways to inside-out integrin activation in diabetic kidney disease.

This research was made possible by the generous support of the Elkins Family Molecular Biology Senior Thesis Fund.

Quantifying the Dynamics of DNA-Promoter Interactions in *Drosophila Melanogaster* Using the Two-State Model

Vishank Jain-Sharma
Advisor: Thomas Gregor

The biological process of DNA transcription in cells, whereby DNA is transcribed to protein-encoding RNA, is the lynchpin to the central dogma, the backbone of all life. While fundamental, DNA transcription is poorly understood, given the process's ubiquity and importance. In particular, the dynamics of the promoter, the region of DNA that initiates transcription for a downstream gene, are not known. The Poissonian model for transcription, which assumes the promoter always remains active, predicts RNA outputs of low variability, yet the evidence strongly suggests, to the contrary, a great variability in RNA production in many genes. The next simplest model, the two-state model, assumes the promoter takes two states, 'on' (transcribing) and 'off' (not transcribing). Through the technique of single molecule Fluorescent In Situ Hybridization (smFISH) in Drosophila embryos, we observe absolute levels of RNA content by analyzing images of the nuclei containing fluorescent spots, each of which, corrected for background noise, represents either a single molecule of RNA attached to fluorescent oligonucleotides bound to a specific gene, or a transcription site containing many of these. Critically, the former allows us to normalize the latter to equivalent numbers of RNA transcripts per transcription site. Through fitting transcription site data of two-color smFISH for a given gene to the two-state model, we extract parameters for the rate of promoter switching between the on and off states, and the rate of transcription of RNA polymerase. Data has hitherto been collected for the Drosophila Gap genes, and Dorsal-Ventral genes are currently in progress. Through analysis of this data, we will extract the aforementioned parameters across these two major categories of genes, and thereby determine, by comparing the values of these extracted parameters with orders of magnitude deemed reasonable by other experiments, whether or not the two-state model is plausible, and whether its parameter variation yields meaningful insight. This will light onto a process fundamental to DNA transcription, allowing us to gain new deep understanding about transcription in Drosophila, and, by hopeful extension, in every known form of life.

This research was made possible by the generous support of the Lewis-Sigler Institute for Integrative Genomics.

Investigating the Role of ROS Signaling and Mitochondrial Dynamics in the Specification of Stem Cells and Primordial Germ Cells

Javier Janbieh
Advisor: Paul Schedl

One group of regulatory molecules that control whether stem cells differentiate into a terminal cell or maintain an undifferentiated character is reactive oxygen species (ROS). ROS affect identity specification by altering proteins involved in pathways that maintain self-renewal. This project undertaken by our lab seeks to investigate the role ROS play in stem cell identity specification and self-renewal maintenance by observing how ROS affect specification in primordial germ cells (PGCs) in Drosophila Melanogaster. PGCs provide a model system for analyzing identity specification, with easily recognizable defects when proper undifferentiated PGCs are not maintained through specification. In an interconnected series of knockdown experiments, PGC migration observed in genetically modified embryos with increased or decreased ROS levels will be gathered and compared to ROS signaling in stem cell systems to characterize the role of ROS signaling in both. We have hypothesized that ROS modulate identity in PGCs in the same manner that ROS modulate identity in stem cells. It appears low baseline amounts of ROS are necessary for PGC and stem cell specification, and as each population approaches differentiation, ROS levels increase. Activating and suppressing pathways to produce migration phenotypes similar to those observed in altered ROS embryos has begun to reveal pathways ROS interact with in PGCs.

It has been established that the mitochondria are the main and constant source of ROS production in stem cells as 0.1-0.2% of O2 consumed through oxidative phosphorylation form ROS via the premature flow of an electron to oxygen. We will also therefore be examining the mitochondrial dynamics of PGCs, mainly investigating whether disrupting mitochondrial fission and fusion has an aberrant effect on proper specification, and seeking to apply that knowledge to earlier data in order to paint a complete picture of the processes at work in stem cell and PGC specification.

This research was made possible by the generous support of the Elkins Family Molecular Biology Senior Thesis.

The Role of Human Satellite Repeat II in Human Cytomegalovirus Infection

Delaney Johnson
Maciej Nogalski
Advisor: Thomas Shenk

Human cytomegalovirus (HCMV) is an opportunistic pathogen that is found globally and causes morbidity in immunocompromised individuals. The virus affects many cellular mechanisms in order to ensure a successful infection. One mechanism viruses have been shown to take is regulation of non-coding RNA (ncRNA). There are many types of ncRNA including miRNA, tRNA, and long non-coding RNA (lncRNA). Here, we describe the role of another type of ncRNA, satellite repeats, which have been shown to be overexpressed in cancer and have an immunostimulatory effect. By implementing a whole transcriptome analysis to create an expression profile of satellite repeats, we found that human satellite repeat II (HSATII), was the highest expressed repeat in HCMV infected cells. HSATII was the same repeat found with the highest expression in cancer cells. To monitor the kinetics of HSATII expression, we used a quantitative PCR assay and found that HSATII expression is more robust but slower in fibroblasts compared to its expression in epithelial cells. Moreover, we demonstrated that viral gene expression occurring before viral DNA synthesis is necessary for elevated HSATII expression. To determine the role of HSATII in HCMV infection, we employed a locked nucleic acid (LNA)- based knockdown of HSATII or transient HSATII expression. We found that the decrease of HSATII transcript levels led to a decrease in release of infectious progeny virus, and infection was enhanced by HSATII overexpression. Furthermore, our study showed that HSATII triggers expression of type I interferons and interferon- stimulated genes, and it regulates levels of secreted cytokines. Together, these results suggest that HSATII overexpression is important for efficient HCMV infection. Future research includes expanding to different cell lines and exploring the mechanism of HSATII upregulation. Furthering this study will shed insight into the pathways induced by HCMV and could be useful in designing a therapeutic target.

This research was made possible by the generous support of the Elkins Family Molecular Biology Senior Thesis Research Fund.

Roles of RpoS and efflux pumps in strengthening the permeability barrier of stationary phase *Escherichia coli* cells

Angela Y. Kim
Angela M. Mitchell
Advisor: Thomas J. Silhavy

The outer membrane (OM) of Gram-negative bacteria, like *Escherichia coli,* serves as a permeability barrier against many stressors. When a nutrient is depleted, cells enter stationary phase and undergo changes that allow survival in stressful conditions. Depending on the nutrient limited, the AcrAB-TolC efflux pump may export harmful compounds, or an alternative sigma factor RpoS may cause gene expression changes strengthening the OM. SDS sensitivity can be used to model these changes. Previous studies have suggested carbon-limited cells primarily use RpoS to survive SDS treatment, while nitrogen-limited cells can use either RpoS or efflux pumps. Based on these findings, we studied the effects of limiting another nutrient, sulfur. Wild-type sulfur-limited cells did not decrease in viability during SDS treatment, while deletion of *acrA* or *rpoS* resulted in a 42-fold or 133-fold decrease in viability, respectively, in 24 hours. *acrA rpoS* double mutant cells showed a greater reduction in viability (700,000-fold in 24 hours) than either single mutant, suggesting that both efflux pumps and RpoS are needed to survive SDS treatment in sulfur-limited conditions, and they can partially compensate for each other when one is lost. To determine the mechanism of RpoS-mediated SDS resistance, *acrA rpoS* double mutant cells that are able to survive SDS treatment under carbon-limiting conditions were isolated. We are currently identifying and mapping suppressor mutations in these cells to find novel pathways or factors involved in stationary phase OM strength. Understanding changes to resistance mechanisms in differing growth conditions may aid in future antibiotic discovery efforts.

This research was made possible by the generous support of the Elkins Family Molecular Biology Senior Thesis Fund.

Identification of host factors in whole genome wide loss of function screens that confer resistance to yellow fever virus infection

David Kim
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Advisor: Alexander Ploss

Yellow Fever Virus (YFV) is a prototype member of the genus *Flavivirus.* Arthropod-borne flaviviruses, such as zika virus, west nile virus, and dengue virus, are causes of major health and economic concerns worldwide. However, the host determinants of flavivirus infection still remain poorly characterized. Displaying both high replication level and cytopathic effects in vitro, the YFV-17D strain represents an excellent experiment system to identify novel host determinants regulating flavivirus infection. Here, we are taking advantage of the CRISPR-Cas9 system, an RNA-guided nuclease system that induces a double-stranded break at a specific genomic locus, to knock out more than 20,000 individual genes and 2,000 miRNAs expressed in a hepatoma cell line susceptible to YFV-17D infection. Following a successful amplification of cells resistant to YFV-17D infection, we sorted individual resistant cells via FACS single cell sorting and confirmed the resistance of the clones through a round of second infections. We isolated one hyper-resistant clone, 5E6, which displayed limited cytopathic effect, impaired intracellular viral replication, and a defect in viral particle release. We are in the process of identifying the knocked out protein in 5E6 through multiple approaches including deep-sequencing and sanger sequencing, prior to the characterization of its pro-viral function. The role of this host determinant in the replication cycle of other flaviviruses will also be investigated.

This research was made possible by the generous support of the Princeton Grand Health Challenge Program.

Temporal Sequence Learning Patterns in the Mouse Primary Visual Cortex Microcircuit

Hyewon Kim
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Advisor: Michael J. Berry

We are able to perceive the visual world because the open eye never completely stops moving as temporal sequences are transmitted to the six layers of the cortical microcircuit. Among these are layers 2 and 3 (L2/3), which in the mouse have been shown to receive input from layer 4 (L4). What we do not know is how this interaction is related to the previously discovered “simple” and “complex cells” in the cat visual cortex that responded to orientation stimuli at differing spatial ranges. We hypothesize that L2/3 register a string of stimuli from L4 as a temporal sequence, similarly to how complex cells may spatially integrate single orientation stimuli. Through two-photon calcium imaging of the Thy1-GCaMP mouse primary visual cortex (V1), we found that when we showed sequences of orientation pattern frames, and recorded the activity of L2/3 neurons, they showed an adaptation, or learning, response that we could fit to an exponential curve. We were then able to generate a time constant for the average adaptation block of each sequence length. We also found that when we introduced a violation frame, the neurons’ violation response became stronger over multiple days, suggesting learning. To perform the same experiments on L4 cells, we first needed to see if the mouse strain that we had previously used had L4 cells labeled, and found that it did not. Through 3D visualization analysis of a z-stack of the Ai93 mouse cortex, we are currently determining whether this new strain does. When we perform the experiments, we expect to see in L4 what has been observed in simple cells: a stimulus-specific, rather than L2/3’s sequence-specific, adaptation pattern. By examining the temporal sequence learning patterns of L2/3 and L4 neurons of V1, we will be able to better understand the workings of the canonical cortical microcircuit.

This research was made possible by the generous support of the Elkins Family Molecular Biology Senior Thesis Fund.

Identifying Memory Task Behavior Variation in *C. elegans*

Jihoon Kim
Advisor: Coleen T. Murphy

Despite being genetically identical, individual nematodes within an isogenic population demonstrate a reproducible variation in memory behavior. A small fraction of the nematodes demonstrates an enhanced memory performance by travelling immediately to the test odorant, one hour after the population has been conditioned to positively associate butanone with food. These nematodes, labeled high memory performance (MP) subpopulation, when collected and re-tested for memory, also demonstrated extended memory retention, showing a significant bias toward the test odorant relative to the remaining population, Here, we begin to characterize the transcriptome differences of the High MP and the remaining population, to identify genes responsible for the enhanced memory performance and extended memory retention using a RNA Gene Expression Microarray. Preliminary data shows significant gene differences between the two populations, but due to possible unwanted gene transcription by the paralyzing agent sodium azide used to collect the worms, additional microarrays should be performed on samples collected without the chemical.

This research was made possible by the generous support of the Lewis-Sigler Institute for Integrative Genomics and the Elkins Family Molecular Biology Senior Thesis Fund.

Solving TUT2: An analytical and crystallographic characterization of a non-canonical human PAP

David Kolet-Mandrikov
Advisor: Alexei Korennykh

The objective of my research to obtain a 3-dimenstional structure of human TUT2, a cytoplasmic Poly(A) Polymerase of RNA. It is a little understood protein that functions in a variety of physiological systems, ranging from development of *C. elegans* to the formation of long-term memory in *Drosophila* and HepC resistance in the human liver. The undergraduate who came before me, Kwaku, began crystallization by making a soluble construct with a deletion of 155 N-terminal residues, affecting no conserved domain. We are synthesizing a full length protein and three truncated active site mutants (two of which were done by Kwaku) to study the full length protein and assay the differences between the two to determine activity and potential mechanisms for activation. Of interest is the mechanism of activation. Preliminary results show that the truncated version is active on its own, unlike GLD2 (the *C. elegans* homolog). If the full-length protein is inactive, the N-terminus may function as a negative regulator and could be inhibited by a protein such as GLD3, the partner protein to GLD2. Further assays seek to discover the RNA binding ability of TUT2, of interest because of its architectural similarity to another protein studied in the Korennykh lab, RNase L, an enzyme capable of binding and cleaving RNA. Ultimately, we will characterize TUT2 and obtain a three dimensional structure, allowing us to gain insight into the negative regulatory function of the N-terminus, which has no homology and the structure of which cannot be consistently predicted by computer algorithm.

This research was made possible by the generous support of the Elkins Family Molecular Biology Senior Thesis Fund. I would like to extend my most sincere gratitude to the Elkins family for their financial contribution, Jesse Donovan for his mentorship, Kwaku for laying out the groundwork of my project, and Alexei Korennykh for welcoming me into his lab.

Comparison and ranking of MEK mutations in network meta-analysis of their effects on early zebrafish morphogenesis

Iason Kountouridis
Advisor: Stanislav Y. Shvartsman

Many developmental processes in organisms are controlled by a signaling pathway called the RAS / ERK Pathway. Documented mutations in the gene coding for MEK, one of the central protein components of this pathway, have the phenotypic effect of elongated embryo shape in zebrafish 12 hours post fertilization, whereas wild-type embryos have spherical shape. For a selection of these mutations we quantified their effect on zebrafish embryo elongation by approximating each embryo as an ellipse and measuring its aspect ratio. We next wanted to compare and rank each mutation according to its effect on increasing the aspect ratio of embryos. In a network meta-analysis framework, we employed the Surface Under the Cumulative Ranking curve (SUCRA) model to determine the probability of a given mutation causing the most increased aspect ratio, or more generally having a specific rank in aspect ratio increase. In the context of this probability model, we used our data to derive discrete probability distributions for the effect of each mutation on aspect ratio, then computed the cumulative distribution function (cdf) for each such distribution. The SUCRA value for each mutation was then computed as the area under its cdf curve, which is a number between 0 and 1 giving the average proportion of mutations with smaller effects than the given mutation. This ranking method can also aid in grouping mutations together according to the statistical similarity of their effect, which is the next step in improving our understanding of the differences between MEK mutations and their effects.

This research was made possible by the generous support of the Lewis-Sigler Institute for Integrative Genomics.

Characterizing the Interaction between Sec1/Munc18 Protein Vps45 and Qa-SNARE Tlg2

Kelly Lau
Frederick Allen, Gregory Shimamura
Advisor: Frederick Hughson

Vesicles carry materials between cellular compartments in cell trafficking. The fusion of vesicles to target membranes is a critical step in ensuring delivery of materials to the correct compartment. Vesicle fusion requires SNARE proteins, which are located on the vesicle and target membranes. The formation of a four-helix bundle between the SNAREs of the membranes pulls the membranes together for fusion. Thus specific and efficient vesicle fusion involves regulation of assembling the SNARE complex. Sec1/Munc18 (SM) proteins have been proposed as important factors in the formation of SNARE bundles. Investigating the binding between SM proteins and SNAREs provides insight on the role of SM proteins in vesicle fusion. Syntaxins, a class of conserved target membrane SNAREs, bind to their respective SM proteins with different domains and conformations. Syntaxin Tlg2 has been reported to bind Vps45, a SM protein involved in the trafficking between the Golgi and endosomes. However, it is unclear which mode of binding these proteins exhibit. We are characterizing the interaction between syntaxin Tlg2 and Vps45 using proteins from thermophile *Chaetomium thermophilum* in biochemical and structural studies. Binding assays suggest that Vps45 binds to the cytoplasmic domain of Tlg2, which appears to aggregate into multimers. Future aims to further investigate the interaction include obtaining the crystal structure of the Vps45 and Tlg2 complex and identifying the domains of Tlg2 needed for binding to Vps45. Determining the structural relationship between Tlg2 and Vps45 will offer an understanding of a mechanism underlying the binding between SM proteins and syntaxins during SNARE complex assembly.

This research was made possible by the generous support of the Evnin '62 Molecular Biology Senior Thesis Fund.

Dynamics of the Time-Dependent Ras-to-STAT3 Signaling Circuit with Optogenetics

Jennifer Lee
Advisor: Jared Toettcher

Every cell in our body receives and interprets signals from its neighbors and the environment. However, in many cases, we do not know what triggers signals to be sent by the "sender" cells or how they are decoded by "receiver" cells. One of these cell-cell communication circuits is the recently discovered Ras-to-STAT3 circuit, where activity of one pathway (Ras) induces the secretion of soluble factors that activate a second pathway (STAT3) in nearby cells. Both Ras and STAT3 play key roles in cancer progression and the tumor microenvironment, suggesting that targeting this signaling circuit may be relevant in cancer therapy.

Here, we aim to interrogate the properties of the Ras-to-STAT3 circuit with high resolution, using an optogenetic (light-inducible) tool developed by the Toettcher lab. In cells engineered to express this “Opto-Ras” system, exposure to red light activates Ras, and exposure to infrared light reverses this activation; light has no effect on normal cells. Using this system, we propose that Ras activation initiates two processes that are offset by time. On one time scale, Ras-activated “sender” cells secrete an IL-6 family ligand that activates STAT3 in the neighboring “receiver” cells, a delayed paracrine activation circuit. Interestingly, the Ras-activated “sender” cells exhibit STAT3 inhibition, an intracellular desensitization circuit. Over a time course, the paracrine activation begins after 60 minutes and plateaus about 90 minutes after continuous Ras activation. Strikingly, STAT3 activation occurs only with continuous stimulation, acting as a persistence detector.

Future work will include investigating whether the delay is in transcription, translation, is post-translational, or during secretion. Demonstrating that differential dynamics of Ras signaling can result in various downstream outputs may elucidate how how cells integrate time-varying signals, a process that may be manipulated by cancer.

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A Snail-activated downregulator of Bazooka in the Drosophila early embryo

Joan Y. Lee
Mo Weng
Advisor: Eric F. Wieschaus

Downregulation of cell-cell adhesion and polarity are crucial events in epithelial-mesenchymal transition (EMT), a process that generates tissue diversity during development and is hijacked in pathological conditions such as tumor metastasis. The transcriptional factor Snail is a master regulator of EMT in many systems, including the mesodermal cells of early Drosophila embryos. Snail has traditionally been thought to downregulate adherens junctions (AJs) through direct transcriptional repression of E-Cadherin. Previous research has found that Snail is involved in the post-transcriptional regulation of AJs in coordination with myosin-dependent tension to time the progression of EMT. Recent findings from our lab suggest that Snail-dependent AJ downregulation in mesodermal cells takes place through loss of the polarity protein Bazooka (Baz, Drosophila Par3). We hypothesize that Snail indirectly downregulates Baz through activation of its transcriptional target genes, which in turn downregulates junctions. We have identified a region on the X chromosome that is necessary for normal Baz downregulation in Drosophila mesodermal cells. Further experiments to resolve the gene target are in progress.

This research was made possible by the generous support of the Pitts '63 Molecular Biology Senior Thesis Fund.

Pairwise Associations Between Adjacent Cys_2His_2 Zinc Finger Domains

Michael Lee
Anton Persikov
Advisor: Mona Singh

Transcriptions factors are proteins that control which genes are turned on or off in the genome. As the largest class of eukaryotic transcription factors, Cys_2Hys_2 zinc fingers (ZF) are of primary interest to gain insight on understanding the regulatory mechanisms driving key biological processes. The canonical model for ZF protein-DNA interactions consists of only four amino acid-nucleotide contacts per domain. This model has been the basis for several efforts to predict DNA-binding specificities. When two ZF domains are adjacent their binding specificities overlap, therefore, to better understand ZF binding we perform a systematic analysis of ZF domains that are adjacent. Looking across Human, Mouse, Zebra Fish, Fruit Fly, and Yeast, we find that domain pairing is non-random and preferential pairing is evident.

This research was made possible by the generous support of the Department of Molecular Biology and the Lewis-Sigler Institute Program for Diversity and Graduate Recruitment.

Temporal-Spatial Dynamics of Cellular Commitment During the Epithelial-Mesenchymal Transition

Daniel D. Liu
Toni Celià-Terrassa, Caleb Bastian, Brian Ell
Advisor: Yibin Kang

The epithelial-mesenchymal transition (EMT) is a key step during cancer metastasis, endowing epithelial tumor cells with migratory and invasive capabilities, thus allowing for dissemination of tumor cells through the body and the formation of distant site metastases. Among the pathways capable of triggering EMT is that of the transforming growth factor beta (TGFβ), a signaling protein known to be secreted within the primary tumor environment. Previous research into the TGFβ pathway has shown that two downstream components, miR-200 and ZEB1, engage in a double-negative feedback loop to mediate EMT response. The temporal-spatial dynamics of EMT and its biological consequences, however, remain poorly characterized.

In this study, we build a mathematical model of TGFβ-induced EMT, predicting the transition to exhibit bistability via a hysteretic switch. These theoretical predictions were confirmed experimentally in cultured cell lines, in which we observed a bimodal distribution of epithelial and mesenchymal-like cells within a range of TGFβ input, measured by E-cadherin (E-cad) expression. *In vivo* experiments demonstrated that these dynamics strongly affect metastatic efficiency in mice. To further validate the model, we used CRISPR/Cas9 to delete the ZEB1-binding site (Z-box 2) of the miR-200c promoter, thus eliminating the repression of miR-200 by ZEB1. In these mutant cells, we observed a gradual loss of Cdh1, rather than a bimodal distribution as seen in parental cells, offering further validation of the model. These findings illustrate the cellular dynamics of the EMT program and further elucidate its functional relevance on metastasis.

This research was made possible by the generous support of the Lewis-Sigler Institute for Integrative Genomics.

Unsupervised Quantification of Mouse Behavior

Jason Manley
Ugne Klibaite
Advisor: Joshua Shaevitz

A lack of standardized and high-throughput methods for behavior quantification has long been a difficulty in the study of animal behavior. We have shown that a method developed by Gordon Berman and colleagues (J. R. Soc. Interface 11(99), 2014) for the unsupervised quantification of behavior in the fruit fly *Drosophila* can be similarly applied to the study of mouse behavior. Utilizing a data-driven approach in which behavioral definitions emerge from the structure of postural movement data, rather than *a priori* human definitions, we provide a high-throughput method for quantifying the behavioral palette of the mouse. The animal's behavioral repertoire is visualized in a two-dimensional space representing all the spontaneous behaviors performed by a mouse, and comparing the distribution of behaviors for animals under various conditions allows for the precise quantification of behavioral differences across conditions, genotypes, and time. In combination with modern neural recording technologies, which allow for the measurement of neural activity in freely-behaving animals, our behavioral paradigm will provide the ability to make tangible and meaningful connections between brain activity and behavioral output in the mouse, a prominent model organism in neuroscience research.

This research was made possible by the generous support of the Lewis-Sigler Institute for Integrative Genomics.

Characterizing *Drosophila* Courtship Song Repertoire: Implications for Behavior and its Neural Substrates

David Mazumder
Jan Clemens
Advisor: Mala Murthy

Social interaction provides an environment rich in diverse sensory cues and demands specific motor responses. In *Drosophila,* males court females by producing songs in bouts of pulse and sine modes, and this behavior provides a simple system in which to study how neural circuits integrate sensory information to choose and encode motor decisions. Courtship and fly song can be precisely quantified in custom recording chambers that allow for naturalistic courtship, and males have been found to vary song features like pulse amplitude and bout length according to factors such as female distance, speed, and angle. Given these insights, in conjunction with recorded neural activity, song structure has been used as an output measure for the song production pathway to inform models of the underlying neural computations. Recently, two pulse subtypes have been discovered in *D. melanogaster,* indicating that the song pathway must be able to encode a greater behavioral repertoire than previously thought. In addition, males produce more P2 pulses the greater their distance to the female, suggesting these types carry functional meaning. We found that silencing the wing motor neuron ps1, which was previously shown to affect pulse carrier frequency, slows P1 pulses, while P2 pulses remain unchanged. This result indicates that the song production circuit physically separates the pathways triggering P1 and P2 pulses at least one synapse upstream of the final motor actuators. Future work will investigate the location of the divergence in the song production circuit and the sensory inputs responsible for this differentiation.

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An Investigation into the Degradation of Eicosanoyl-5-hydroxytryptamide (EHT)

David J. McFall
Scott L. Melideo
Advisor: Jeffry B. Stock

Alzheimer's disease (AD) and Parkinson's disease (PD) affect over six million adults in the United States alone. Both diseases have characteristically hyperphosphorylated proteins, which are thought to be responsible for neurodegeneration. Eicosanoyl-5-hydroxytryptamide (EHT), a compound isolated from coffee, has been shown to reduce protein phosphorylation and prevent cytotoxicity in a number of models of AD and PD. EHT is an anti-inflammatory, an anti-oxidant, and a modulator of protein phosphatase 2A (PP2A), the major phosphatase in the brain. These properties support the idea that EHT could be a therapeutic agent for neurodegenerative diseases like AD and PD. Previous studies have shown that extracts from mammalian tissues were capable of synthesizing EHT. This study aims to begin characterizing the degradation of EHT in murine tissues. The C16 equivalent of EHT, palmitoyl-5-hydroxytryptamide (PHT), was used for preliminary hydrolytic assays, as it is found in lower concentrations *in vivo*. Preliminary work focused on developing an assay to measure PHT hydrolysis. The current system uses upright paper chromatography to observe [^{3}H]-serotonin production. The enzyme responsible for PHT degradation was originally modeled after NAD^{++}-dependent deacetylase sirtuin 2 (SIRT2), since recent literature suggests SIRT2's ability to hydrolyze larger fatty-acyl chains and its link with neurodegenerative diseases. Both purified recombinant SIRT2 and murine crude extracts, however, were unable to hydrolyze PHT. Future work will extend the *in vitro* assay using different extraction protocols and mammalian tissues. Ultimately, this project aims to isolate and identify the enzyme responsible for PHT degradation.

This research was made possible by the generous support of the Elkins Family Molecular Biology Senior Thesis Fund.

An in vivo characterization of podosomes and their role in heart morphogenesis in zebrafish

Josh Morrison
Victoria Patterson
Advisor: Rebecca D. Burdine

Cell migration is a necessary biological process that allows events including immune response, organ morphogenesis during embryonic development, and cancer metastasis to occur. Therefore, a defect in cell migratory behavior during the morphogenesis of an organ, like the heart, can lead to serious abnormalities, such as congenital heart defects (CHDs). CHDs represent the largest group of congenital defects that are diagnosed either prenatally or during infancy. The zebrafish is an excellent model for heart development because related genes are conserved across vertebrates. During zebrafish heart morphogenesis, there are two major asymmetric cell migratory events, called cardiac jogging and looping. Nodal signaling drives these asymmetric movements in the heart, but the mechanisms underlying this are unknown. We preliminarily identified Fgd1 as a Nodal signaling target in the zebrafish heart. This protein has been implicated in the formation of podosomes; actin-rich structures that we observe to form during cardiac jogging. Podosomes have not been well characterized, but they are hypothesized to play a role in cell migration due to their up-regulation in metastatic cell lines. The goal of this research is to characterize podosomes in vivo and characterize the role of Fgd1 and other Nodal regulated genes in podosome formation and regulation. Thus far we have begun to generate Fgd1 and Tks5 knockouts in F0 fish. Once they have sexually matured, we can test for germline transmission of the mutation, and hopefully use live-cell imaging and immunostaining to see the effects of these knockouts. We've also started using morpholino knockdown of Tks5, which have caused delays in cardiac jogging, indicating abnormalities in cell migration.

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Thermoregulation of the CRISPR-Cas Adaptive Immune System in *Pseudomonas aeruginosa*

Katrina A. Muñoz
Nina M. Hoeyland-Kroghsbo
Advisor: Bonnie L. Bassler

CRISPR-Cas (Clustered Regularly Interspaced Short Palindromic Repeat) is an adaptive immune system that enables prokaryotes to detect and destroy infecting DNA. This system includes the crucial step of adaptation, which involves the cleavage and incorporation of foreign DNA sequences into the CRISPR locus. These sequences, known as spacers, are located between direct repeats. After adaptation, processed crRNAS (CRISPR RNAs) guide Cas protein complexes to complementary foreign DNA sequences that are then cleaved, resulting in immunity. Although considerable research has been conducted on the mechanism of CRISPR-Cas, very little is known about how the system is regulated. Adaptation was investigated in the pathogen, *Pseudomonas aeruginosa* PA14. Adaptation occurs by acquisition of spacers causing an expansion of the CRISPR locus, which was measured by PCR. Primers that bind upstream the first repeat and inside the second spacer of the parent CRISPR locus were utilized for PCR, and fragments were separated on an agarose gel for visualization of the spacer population. Different plasmids were tested for their ability to promote adaptation. A plasmid containing a protospacer directly targeted by CRISPR but containing one adaptation promoting seed mutation was chosen for experimentation. Adaptation was also promoted by growth on plates rather than in a liquid medium. When compared to growth at 37°C, colder temperatures of 30, 23, and 15°C enhanced adaptation, with 15°C promoting the highest degree of adaptation. In addition, quorum sensing, or cell-to-cell signaling, was previously found to increase adaptation. However, this positive effect of quorum sensing on adaptation was here found to be more pronounced at 23 and 30°C compared to 37°C.

Little is known about the regulation of CRISPR-Cas, however this study identified three important physiological regulators of adaptation: growth on solid surface, temperature, and a synergistic effect of quorum sensing and temperature.

This research was made possible by the generous support of the Genentech Foundation.

Improving FNTM: a server for predicting functional networks of tissues in mouse

Sara Myers
Jonathan Goya
Advisor: Olga Troyanskaya

Complex genetic diseases have multiple genetic and environmental factors, none of which are themselves solely causative. When searching for genes which may contribute to a certain phenotype, it is often unrealistic from both a cost and a time perspective to test each gene in the genome. With a smaller subset of genes which have a greater probability of contributing to that phenotype, testing those genes becomes more feasible. We aim to create a probabilistic model of the functional network of tissues in the mouse. Using Bayesian integrations and standards of gene interaction gathered from functional genomic data, we predict the probability that two genes interact in different tissues in the mouse developmental anatomy ontology (EMAPA). We then test the performance of the model in mapping gene interactions both globally and in each tissue, generating receiver operating characteristic curves to evaluate performance. We also generate precision-recall curves when evaluating the performance of the model in predicting a gene's role in the expression of a phenotype. We have found that the model's performance has a negative correlation with both the number of edges and to the ratio of positive gene interactions to total gene pairs in the training data. Additionally, we have found no evidence that the model's performance varies depending on the branch of the ontology. Results show that some tissues perform better than others when predicting whether a gene contributes to the expression of a tissue-specific phenotype. We will explore reasons that the model's performance might be affected by the number of edges and the ratio, as well as why some tissues perform better in phenotype predictions, in order to improve its accuracy. In the future, this model can be used to identify new genes which may contribute to the expression of a phenotype that may be better targets for drug treatments.

This research was made possible by the generous support of the Department of Molecular Biology and the Lewis-Sigler Institute Program for Diversity and Graduate Recruitment.

Characterizing MHCI cytoplasmic domain phosphorylation and its role in binding to spinophilin

Emily E. O'Driscoll
Karla K. Frietze
Advisor: Lisa M. Boulanger

Proteins of the major histocompatibility complex class I (MHCI) are well known for their involvement in the immune system. More recently, studies have identified unexpected, essential functions for MHCI at synapses within the mammalian brain. MHCI negatively regulates synaptic transmission mediated by N-methyl-D-aspartate receptors (NMDAR), glutamate receptors essential in the establishment and function of neural circuits. MHCI is important for NMDAR-dependent forms of plasticity, learning, and memory. Given that MHCI's effects on NMDAR function are so large in magnitude and appropriate NMDAR function is critical to normal brain function, it is likely that MHCI's ability to regulate NMDARs is itself tightly regulated. However, little is known about the molecular mechanisms by which MHCI regulates NMDARs or how this regulation could be dynamically controlled. In the current study, I built on preliminary results from the Boulanger lab suggesting that this NMDAR regulation occurs via PDZ protein-protein interactions with MHCI's short cytoplasmic domain (CD), specifically addressing the possibility that dynamic phosphorylation of serine, threonine, and/or tyrosine residues within the MHCI CD rapidly regulates MHCI's binding interaction with the synaptic protein spinophilin. A better understanding of how MHCI-PDZ binding is regulated would help uncover the precise mechanism of MHCI regulation and signaling in the healthy and injured brain.

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Investigating the Role of the Pseudorabies Virus (PRV) Tegument Protein VP13/14 in Inhibiting Host IFN-β Response

Joshua Payen
Margaret A. MacGibeny
Advisor: Lynn W. Enquist

Pseudorabies virus (PRV) is an alpha herpesvirus native to pigs. PRV and other alpha herpesviruses such as herpes simplex virus (HSV) and Varicella-Zoster (VSV), infect the periphery nervous system then establish a latent infection in the nucleus allowing for future reactivation. The interferon-β (IFN-β) pathway, which helps to establish an antiviral state within the host cell, must be overcome before the virus can establish a productive or latent infection. For example, HSV uses several proteins to mediate the innate immune response. Mediation by PRV is much less well understood particularly because PRV lacks homologs for many of these proteins. This study investigates the role of the UL47 gene product, VP13/14, in mediating the IFN-β response in host cells. This abundant outer tegument protein is hypothesized to bind phosphorylated STAT1, a transcription factor needed to promote IFN response, thereby sequestering it to cytoplasm and/or targeting it for degradation. Using UL47 knockout mutants (ΔUL47) generated by the CRISPR/Cas9 system, this study will measure the effect of VP13/14 on pSTAT1 levels and also visualize changes in pSTAT1 localization due to VP13/14. This study will determine how the virus interacts with the host IFN response, which could improve the way herpes viral infections are treated.

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Investigating the LPS Transport Role of LptE as a Member of the LptD/E Complex in *Escherichia coli*

Vikram Pothuri
Marcin Grabowicz
Advisor: Thomas Silhavy

Gram-negative bacteria are a ubiquitous class of bacteria that can cause a number of refractory infections. These bacteria are able to flourish in harsh environments and are highly resistant to antibiotics, in large part, because of their unique cell envelope configuration. Their envelope is made up of two membranes that are separated by an aqueous periplasmic space. The outer membrane is an asymmetric bilayer with a molecule called lipopolysaccharide (LPS) being responsible for the membrane's fundamental permeability barrier function. LPS is transported from the cytosolic face of the inner membrane, through the periplasm, and to the outer membrane by the Lpt pathway. While many steps in this pathway are well characterized, there is uncertainty surrounding the way in which the LptD/E complex likely guides LPS into the outer leaflet of the outer membrane. LptD is an integral β-barrel protein and LptE is a lipoprotein that interacts with and resides within LptD. I am conducting two separate, but interrelated approaches with the intention of identifying and characterizing LptE residues that participate in initial LPS interaction, and therefore are essential in LPS transport. I have mutated residues on LptE's middle β-strand, and have conducted antibiotic sensitivity screens in diploid and haploid backgrounds. So far, I have identified two promising mutations, T103R and E97R, which show dominant, growth and antibiotic sensitivity phenotypes respectively. Next, I will vary the LPS substrate that these mutant LptE proteins encounter and look for changes in viability, growth and antibiotic sensitivity. This will help to uncover additional LptE residues important for LPS transport as well as to better characterize the regions of LPS that participate in LptE interaction.

This research was made possible by the generous support of the Rupert and Loretta Jones Molecular Biology Senior Thesis Fund.

The Enzymatic Synthesis of Eicosanoyl-5-hydroxytryptamide

Audrey Potts
Advisor: Jeffry B. Stock

Neurodegenerative diseases such as Alzheimer's disease (AD) and Parkinson's disease (PD) are characterized by progressive neuronal dysfunction and death caused by abnormal aggregations of hyperphosphorylated proteins in the brain. One mechanism that leads to hyperhphosphorylated proteins is reduced protein phosphatase activity. In the brain, decreased activity of a specific protein phosphatase called protein phosphatase 2A (PP2A) leads to the neuronal abnormalities associated with hyperphosphorylated proteins seen in neurodegenerative diseases. A compound found in coffee called eicosanoyl-5- hydroxytryptamide (EHT) has been shown to provide neuroprotective effects by mediating PP2A function. In order to elucidate EHT's role in the body, I propose that EHT is synthesized enzymatically in the body via a condensation reaction and that the enzyme (EHT synthase) belongs to a class of enzymes called ceramide synthases. I am testing my hypotheses by quantifying EHT production in rat intestinal membrane using a novel enzymatic assay and high-performance liquid chromatography (HPLC). EHT production will be confirmed using mass spectrometry. Preliminary results suggest that EHT production is dependent on the presence of eicosanoyl-CoA (E-CoA) and serotonin (5-HT), and is produced linearly with respect to time in the membrane fraction of rat intestine. Additional work on characterizing EHT synthase is required, which includes verifying the identity of the enzyme. In conclusion, preliminary results suggest that EHT is produced enzymatically in the rat intestinal membrane. This discovery could be very useful for developing novel pharmaceuticals for treating neurodegeneration.

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Developing a Model of Stress-Induced Telomere Attrition in Human Foreskin Fibroblast Cells

Nandita Rao

Iulia Kotenko, Lisa Schneper
Advisor: Daniel Notterman

Telomeres are nucleoprotein complexes that cap the ends of each strand of DNA in order to prevent the loss of genetic information during replication, protect the DNA from degradation, and inhibit chromosome fusion. During replication in most cells, telomeres shorten because of the end replication problem and continue to do so until they reach a critical length, resulting in cellular senescence. Telomere shortening is a natural process; however, individuals exposed to chronic stress have been observed to have shorter telomeres then those who have not. The biological significance and mechanism of stress-related telomere attrition are unclear. In order to establish a model and elucidate the molecular mechanism behind telomere attrition, human foreskin fibroblast cells (HFF) were cultured and exposed to two stressors: cortisol and estradiol. Cells were then harvested for two analyses: FACS and qPCR. Utilizing FACS, the percent of cells in each phase of the cell cycle were quantified. In the estradiol-exposed cells, we preliminarily noted a two-fold increase in the amount of cells in the S-phase. Utilizing qPCR, the enzymatic activity of telomerase was analyzed, based on the amount of double-stranded TTAGGG repeats synthesized during a PCR run. A standard was created from HEK-293T cells that constitutively express telomerase. Estradiol and cortisol were preliminarily shown to cause no effect on relative telomerase activity after correcting for cell cycle delay. Currently, this methodology is being applied to analyze the effects of other mediators of stress, such as TNF-alpha, IL-6, as well as environmental stressors, such as heat and oxidative stress, on telomerase activity and cell cycle progression. Future experiments will quantify the effects on telomere length and changes in the transcriptome to discover potential pathways that may contribute to the molecular mechanism of telomere attrition.

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Characterization and Tuning of Xenopus laevis Nucleolar Dynamics using the Cryptochrome 2 (CRY2-olig) Optogenetic System

Tiffany M. Richardson
Lian Zhu
Advisor: Clifford P. Brangwynne

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease that effects about 350,000 people worldwide. Certain ALS dipeptides have been shown to target a specific ribonucleoprotein body, the nucleolus, and interrupt ribosome biogenesis. The nucleolus is compartmentalized in order to facilitate sequential spatially distinct enzymatic reactions starting with rDNA transcription, then modification/processing, and ending with ribosomal assembly. The way in which biophysical properties effect molecular interactions of the different nucleolar compartments is still unclear. I used optogenetic manipulation of a reversible, blue light induced oligomerizing protein, cryptochrome 2 (CRY2-olig), fused to nucleolar proteins to modulate molecular interactions between nucleolar proteins in the large Xenopus laevis oocyte (≥ 1um) nucleus (~450um). I used fluorescence recovery after photobleaching (FRAP) to characterize the impact on mobility of proteins in the different compartments after activating CRY2-olig with blue light. We have found that CRY2-olig affects inter-compartment dynamics by slowing diffusion rates, reducing fractional recoveries, and changing actin-disruption mediated nucleolar fusion dynamics. More specifically, I have found that CRY2-olig gelation of the granular component may slow the dynamics of the dense fibrillar component when both are co-expressed. Further experiments need to be done to determine whether there is a compartmentally dependent effect on intra-compartmental dynamics and overall nucleolar function. It is hypothesized that slowing dynamics of the inner-most compartment where transcription occurs will subsequently slow the dynamics of the outer compartments due to the sequential nature of rRNA biogenesis. These findings will shed light on the connection between the physiology of the nucleolus as a multi-phase liquid organelle dependent and its function of ribosomal biogenesis and subsequent transfer of genetic information.

This research was made possible by the generous support of the Elkins Family Molecular Biology Senior Thesis Fund, the Genentech Foundation, and the Brangwynne Lab.

Flux maximization through an enzyme budgeted pathway as a model for glycolysis kinetics and enzyme pool sizes in *Escherichia coli*

Iris Rukshin
Zhiyuan Li, Thibaud Thaillefumier
Advisor: Ned Wingreen

Metabolism is the process by which nutrients are converted into energy and biomass. This sequence of complex reactions is facilitated by enzymes produced in specific quantities. To understand the mechanisms involved in metabolism, recent efforts have been applied to map out the reversibility, fluxes, and thermodynamics of metabolic pathways, as well as metabolite concentrations in each step. However, little is known about the determination of the enzyme pool sizes involved in these reactions. Since metabolism is evolutionarily conserved across organisms, a theoretical model for these reactions is highly relevant. This study focuses specifically on glycolysis in *Escherichia coli* and hypothesizes that enzyme pool sizes can be explained by the kinetic optimization of each reaction. Here, a model is proposed for a two-step isomerization reaction with two enzyme substrate complexes in each step that maximizes the flux through the reaction and restricts the concentrations of the two enzymes to a mutual budget. This model was applied to experimental data to predict and justify observed enzyme abundances in *Escherichia coli*. In the future, this model can be expanded and specified to theoretically represent multiple different steps in glycolysis and the metabolic pathway.

This research was made possible by the generous support of the Lewis-Sigler Institute for Integrative Genomics.

Characterization of the MZT in *Ciona* via a Novel Method of Tracking Transcript Origin

Sarah Santucci
Kai Chen
Advisor: Michael Levine

The switch from reliance on maternally-provided mRNAs in the cytoplasm to transcription of the zygotic genome is crucial to development in all animals. The process by which this maternal-to-zygotic transition (MZT) takes place occurs differently, in both timing of gene activation and in which genes are activated, across the metazoans. We are using the simple chordate *Ciona* to understand how these crucial development pathways evolve and function in an organism that is closer on the evolutionary tree to vertebrates than to nematodes and arthropods, organisms that have dominated MZT research. We have developed a novel method for easily identifying transcript origin to precisely characterize the MZT in *Ciona* using the cross *C. savignyi*♂ *x C. intestinalis*♀ and differentiating between maternal transcripts, which only have *C. intestinalis* sequences, and zygotic transcripts, in which *C. savignyi*-specific sequences will be present, using ChIP- and RNA-seq. We are also conducting ChIP-seq experiments for transcription factors in both species and hybrids, which will identify the earliest active promoters and the transcription factors crucial in their activation as well as the timing of their activation. Differences in the two species' gene activation profiles may help elucidate the evolutionary changes in regulatory regions of DNA that lead to speciation. Studying the MZT in the *Ciona* system will lead to insights not only the mechanisms of gene activation but how differences in gene activation arise and result in phenotypic changes. These insights will help us complete the picture of how both vertebrates and invertebrates undergo this crucial early developmental process and how these mechanisms evolved.

This research was made possible by the generous support of the W. Reid Pitts, Jr., M.D. '63 Undergraduate Research Fund.

REGULATION OF GLYCOLYTIC FLUX BY PFK2/PFKFB ISOFORMS

Talen Sehgal
Lukas Tanner
Advisor: Joshua Rabinowitz

Cancer cells often display an altered glucose metabolism, characterised by high glycolytic activity even in the presence of oxygen. This phenotype is known as aerobic glycolysis or the Warburg effect, and it enables the cell to overcome the high demand for energy and biosynthesis required by increased cell proliferation. In order to further understand how glycolysis is regulated, it is important to first understand how the committing steps in glycolysis are regulated. Phosphofructokinase-1 (PFK1) is a well-known rate-limiting enzyme which is regulated by its allosteric activator, fructose-2,6-bisphosphate (F-2,6-P_2). F-2,6-P_2 levels are regulated by 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase (PFK2/PFKFB1-4) isoforms along with the bisphosphatase TIGAR. Independently, the PFKFB1-4 genes either promote production or decomposition of F-2,6-P_2. Our findings reveal an isoform specific hierarchy of glycolytic flux regulation which aligns well with known kinase:phosphatase ratios. Overexpression of PFKFB4 does not exhibit any significant control of glycolysis whereas PFKFB2 decreases glycolytic flux. PFKFB1 enhances flux, but to a lesser extent than PFKFB3, which leads to the highest increase in glycolytic flux control. Coexpression of these isoforms leads to a glycolytic flux control that lies between the level of flux control observed in single overexpression of the PFKFB genes. These findings add substantial knowledge on how different PFK2/PFKFB isoforms regulate glycolytic flux and will eventually guide us to better understand the Warburg effect in cancer cells.

This work was made possible by the generous support of the Lewis-Sigler Institute for Integrative Genomics.

The mechanism of action of SCH79797 and Calmidazolium Chloride

Christian Shema Mugisha
James Martin
Advisor: Zemer Gitai

Antibiotics are useful in modern medicine and in other areas such as agriculture. However, the rise and spread of antibiotic resistance is a threat. The epidemic of antibiotic resistance may be combated through the use of antibiotics with novel mechanisms of action (MoA). Previous experiments in our lab have demonstrated that Calmidazolium chloride and SCH79797 may in fact possess novel MoA. Calmidazolium chloride (Cal) is a drug that inhibits calmodulin and other calcium-binding proteins, while SCH is a potent PAR1 receptor antagonist. Despite the absence of these targets in bacteria, our experiments have shown that both Cal and SCH inhibit bacterial growth. Using an imaging based method of MoA determination known as bacterial cytological profiling (BCP), we discovered that Cal and SCH may potentially have novel MoA. The focus of this project is to identify the MoA of Cal and SCH. We proceeded to identify the molecular target of Cal and SCH by isolating spontaneous *Escherichia coli* mutants resistant to each of the drugs. SCH resistant mutants were acquired and their resistance mutations were confirmed to be stable, inheritable, and partially specific to SCH. In contrast, mutants resistant to Cal were not able to be obtained. We hypothesize that our inability to acquire Cal resistant mutants could be because Cal either has multiple targets, or targets an essential cell component, such as the cell membrane. Preliminary evidence suggests that Cal neither affects membrane structure nor permeability. Additional work will be required to investigate other possible mechanisms of Cal activity. Further characterization of the SCH resistant mutants will also be required. However, if resistance specific to SCH can be obtained, whole genome analysis will be used to identify the mutations that confer SCH resistance, providing insights into the MoA of SCH.

This research was made possible by the generous support of the Department of Molecular Biology and the Lewis-Sigler Institute Program for Diversity and Graduate Recruitment.

Decoding *dod-18*: An RNAi Mediated Approach To Analyzing Expression of A Lifespan Extension Gene

In *Caenorhabditis elegans*

Sunny Siddique
Advisor: Coleen T. Murphy

Our lab is dedicated to studying the complex molecular underpinnings of age-related decline. In this paper, we focus on elucidating our understanding of the DAF-2 signaling cascade, involved in promoting lifespan extension in Caenorhabditis elegans. In the absence of DAF-2 signaling, its downstream transcription factor DAF-16 remains unphosphorylated and nuclearly localized, resulting in the expression of various downstream target proteins that have been shown to regulate longevity in the organism. We are concerned with studying the cellular and sub-cellular localization of dod-18, a class II target gene whose downregulation by DAF-16 has been shown to extend lifespan. Along with the location where it is expressed, the functional implications of dod-18 and its correlation to known age-related pathways continue to be uncharacterized. As a result, we hope to study whether dod-18 or its overlapping neighboring gene C54G4.7 is responsible for lifespan extension. Furthermore, we want to expand our understanding of its localization by using microinjection techniques to express Pdod-18::gfp in the germline and observe in which tissue it is expressed in the transgenic progeny. Finally, we hope to gain insight into the functional aspects of dod-18 by making a translational fusion, DOD- 18:: GFP and looking at the subcellular location where it is expressed.

This research was made possible by the generous support of the Elkins Family Molecular Biology Senior Thesis Fund.

Investigating the Role of Planar Cell Polarity in Orienting the Mitotic Spindle

Jorge Sion
Kimberly Box
Advisor: Danelle Devenport

Planar Cell Polarity (PCP) is responsible for orienting and aligning cellular components along the epithelial plane across whole tissues. This feature promotes the correct alignment and directionality of inner ear hair, ensures the proper beating of cilia in the trachea, and stimulates appropriate orientation of cell divisions during zebrafish gastrulation. The epidermis has been shown to be a tissue in which cell polarity plays an important role for proper functioning, one example being determining the way cells divide. A type of polarity, known as Apical-Basal Polarity, has been shown to regulate asymmetric, perpendicular to the basal layer, divisions by orienting the mitotic spindle, however not much is known about what regulates symmetric, planar to the basal layer, divisions. It is plausible to think that PCP could regulate symmetric divisions by orienting the mitotic spindle similarly to Apical-Basal Polarity. Preliminary data collected from imaging Vangl2 mutant skin explants revealed that symmetric divisions decreased when compared to wildtype skin explants. These findings suggest that Vangl2, a PCP protein, is somehow influencing symmetric divisions. In this study, we aim to confirm that PCP, through Vangl2, can in fact orient the mitotic spindle similarly to Apical-Basal Polarity. Furthermore, once this is established, we aim to elucidate the mechanism behind Vangl2's ability to orient the mitotic spindle. This will be accomplished by determining which proteins localize to Vangl2 during spindle orientation and observing which knockdowns of said proteins interrupt spindle orientation. These findings could help shine some light on an otherwise unknown mechanism that determines cell fates.

This research was made possible by the generous support of the Elkins Family Molecular Biology Senior Thesis Fund.

Investigating Mitochondrial SIRT3 and its Anti-Viral Functions

Morgan Sly
Xinlei Sheng
Advisor: Ileana Cristea

Sirtuins are NAD+-dependent deacetylases found within human cells. Recently, research has shown that sirtuins play a critical role in inhibiting viral replication within the host cell. The mechanisms through which they achieve this function remain unclear. My proposed thesis research will examine the mitochondrial sirtuin 3 (SIRT3), a deacetylase thought to regulate the acetylation levels of numerous proteins with critical roles in cellular metabolism. Specifically, I hypothesize that SIRT3 is able to inhibit viral replication by interacting with factors in host metabolic pathways that are crucial for viral replication. I also hypothesize that SIRT3 uses its enzymatic activity in order to alter its substrates functions and thus confers an anti-viral effect. The virus that I will focus on is the β-herpes virus, human cytomegalovirus (HCMV) because it expresses specific viral proteins at different time points throughout infection. Thus far, the Cristea lab has identified a preliminary list of proteins that interact with SIRT3, both showing significant increases or decreases in its associations with substrates following infection with HCMV. I found that infected cells with knockdown of ACAD10 or CPS1 exhibited healthier morphologies compared to wild type and were also able to reduce viral titer. Overall, this research may increase the potential for taking advantage of sirtuins ability to initiate a potent anti-viral response to a broad spectrum of viral types.

This research was made possible by the generous support of the Elkins Family Molecular Biology Senior Thesis Research Fund.

Determining the Conservation of Sirtuin Lipoamidase Activity in Bacterial Homologs

Caroline Snowden
Advisor: Ileana Cristea

Lipoic acid is a necessary cofactor for several evolutionarily-conserved metabolic enzymes. Its cleavage from these enzymes is an important method of metabolic regulation, but much about this process remains unknown. Our lab has recently identified sirtuin 4 (SIRT4) as the first known mammalian lipoamidase, acting primarily as a negative regulator of the metabolic enzyme pyruvate dehydrogenase. Interestingly, bacteria also possess sirtuin homologs; for example, CobB in *E. Coli* and SrtN in *B. subtilis* closely resemble SIRT4 in sequence homology. My research has aimed to use CobB and SrtN knockout and overexpressed cells to determine whether the lipoamidase activity of SIRT4 is conserved in its bacterial homologs. Our results show that knockout of the bacterial sirtuins CobB or SrtN correlates with elevated cellular levels of lipoylated subunits, while overexpression produces the opposite effect. Sirtuin levels also impact the function of metabolic enzymes, diminishing the activity of PDH and OGDH in CobB or SrtN overexpressed cells. Bacterial sirtuins therefore appear to possess lipoamidase activity that regulates the metabolic enzymes PDH and OGDH via inhibition of their lipoylated subunits. When metabolic state is altered, bacterial sirtuin knockout also delays changes in lipoylation of key metabolic enzymes. Because sirtuins have been demonstrated to possess antiviral properties, this could provide insight into the possible role of sirtuin lipoamidase activity in viral infection, which is associated with changes in metabolic state. Altogether, my study expands the understanding of sirtuins and their role in metabolic regulation during viral infection, assisting future development of antiviral therapies.

This research was made possible by the generous support of the W. Reid Pitts, Jr., M.D. '63 Undergraduate Research Fund.

Role of COPII components in oocyte determination and maintenance in Drosophila melanogaster

Dorothy Tang
Advisor: Gertrud Schüpbach

An important problem in developmental biology is the analysis of how cell fate decisions are made and maintained. The question of how germline stem cells give rise to a mature oocyte is particularly interesting, but it is not well understood how the oocyte identity is determined and maintained in Drosophila melanogaster oogenesis. An ethyl methanesulfonate (EMS) screen was conducted to discover new genes that have roles in oogenesis. In this screen, a mutant line JV91 gave rise to a phenotype of missing oocytes, ring canal clustering, and mislocalization of oocyte-specific factors such as Orb, Bicaudal-D, and Gurken. JV91 was identified as an allele of Sec24CD, which is a component of the COPII secretory pathway. I have obtained results suggesting that other proteins participating in the COPII pathway are also critical in oogenesis. RNAi knockdown of Sar1 and Sec23, both components of COPII, result in very rudimentary ovaries. In ongoing experiments, Sec23-tagged transgenic fly lines are underway to visualize the protein's localization. Mutant clonal analysis of different alleles of Sec23 and Sar1 is also being conducted. In future studies, I will determine whether COPII vesicles transport oocyte-specific factors as cargo to differentiate the oocyte. A closer understanding of the roles of Sar1 and Sec23 will provide further insight into the process of oocyte determination, as well as allow us to discover further mechanisms of the COPII pathway, a pathway that is very much conserved among eukaryotic organisms.

This research was made possible by the generous support of the Elkins Family Molecular Biology Senior Thesis Fund.

Beyond the X: Detecting Cancer Drivers from Non-Coding Regions in Whole Genome Sequencing Data

Xiaodi Tang
Pawel F. Przytycki
Advisor: Mona Singh

With the declining cost of genome sequencing comes a surge in sequencing data for not only populations of various species, but also genomes of normal and diseased cells in cancers. Within the scope of these efforts, limiting analysis to data generated from Whole Exome Sequencing (WES/WXS) has become the general practice. This practice stems in part from reasons of cost efficiency and in part from long-held beliefs that, aside from protein-coding genes, the genome otherwise consists of "junk dna." However, less than 2% of the human genome encodes protein. Non-coding regions have been shown to serve critical roles, ranging from gene regulation to RNA processing, yet are not well studied in cancer research. Considering the dearth of pipelines analyzing non-coding regions—despite their importance—we aimed to develop a pipeline that discovers cancer driving mutations when given matched tumor-normal samples. Most notably, this pipeline incorporates an adapted version of a new cancer driver gene discovery method, Differential Mutation Analysis, shown to outperform other recent methods despite needing fewer parameters as input. Since non-coding driver mutations affect the expression of protein-coding genes regulated by the corresponding non-coding regions, identification of non-coding driver mutations will facilitate therapeutic efforts by recommending target proteins.

This research was made possible by the generous support of the Lewis-Sigler Institute for Integrative Genomics.

Characterizing the Role of Nanos/Pumilio and Matrix Metalloproteinases in the Regrowth of *Drosophila* Class IV Dendritic Arborization Neurons

Sajal Tiwary
Advisor: Elizabeth R. Gavis

Neuronal function fundamentally depends on proper dendrite morphogenesis, as many clinical disorders arise from abnormal morphology. Understanding novel dendritic regrowth mechanisms can elucidate the regulation of both initial morphogenesis and later regrowth. *D. melanogaster* dendritic arborization (da) neurons serve as good models for these phenomena. Larval class IV da neurons possess complex arbors, which are pruned and regrown during pupation. The molecular regulation of this regrowth is poorly understood. We investigated the roles of larval class IV da neuron branching, tiling, and remodeling factors in adult neuron morphogenesis by GAL4-UAS RNAi. Among these, only *pumilio* knockdowns produced a consistent phenotype; in two-day old adults, under-branched class IV da neurons were observed. Although Pumilio forms a complex with Nanos to regulate larval morphogenesis, no corresponding phenotype was observed for *nanos* knockdown. However, both *nanos* and *pumilio* knockdowns showed a defect in the distribution of dendrites in two-day old adults. Immunofluorescence analysis showed that Nanos levels are slightly increased throughout pupation. These results indicate possibly independent roles of Nanos and Pumilio in adult class IV da neuron morphogenesis. Analogous knockdown of *matrix metalloproteinase 1* (*mmp1*) and *mmp2*, previously implicated in class IV da neuron dendritic remodeling, produced no consistent branching or dendritic distribution defects. This suggests a role for the Mmps outside of the neuron in mediating dendritic remodeling. Pupal-specific knockdown and overexpression of these four factors will be done using the $GAL80^{ts}$/GAL4-UAS system, followed by MARCM analysis to ensure that these effects are cell-autonomous.

This research was made possible by the generous support of the Class of 1943 Molecular Biology Senior Thesis Fund.

Investigating Localization of Prickle1, Prickle2, and Prickle3 Genes in Electroporated Transgenic Mouse Embryos

Narlyn Veliz
Bradley Joyce
Advisor: Danelle Devenport

Planar cell polarity (PCP), or the property that accounts for the orientation and alignment of cells within the plane of an epithelial sheet, is dependent on a network of PCP proteins that transmit the signals needed to coordinate the local and global polarization of cells. It has been shown that PCP plays a role in hair follicle morphogenesis in the embryonic epidermis of the mouse, and the phenotypic effects of specific PCP mutants include whorled hair patterns and misaligned hair follicles. Prickle is a core PCP protein that has been associated with the transmembrane PCP protein Vangl2 on the anterior side of cells. Three Prickle homologs have been identified in mammals, however their localization in the basal layer has not yet been assayed. Studying PCP in embryonic epidermal cells can sometimes require transgenic mouse lines, which are predominantly created using pronuclear microinjection. However, this procedure is tedious and labor intensive as each zygote has to be individually injected. In this study, we plan to use electroporation instead to create transgenic mouse embryos, which would allow for earlier screening and higher throughput of gene transfer than pronuclear microinjection. As a proof of principle, we have been working on generating three GFP-tagged Prickle constructs that will later be introduced into pre-implantation embryos using electroporation as the method for DNA delivery. This will not only bring a much-needed technology for genetic manipulation to the lab, but will also provide insight on the localization of three understudied, but essential, Prickle homologs.

This research was made possible by the generous support of the Elkins Family Molecular Biology Senior Thesis Fund.

Automating and batch-processing of imaging data analysis facilitates the examination of transcriptional dynamics at the single nuclei level in the early Drosophila embryo

Samvida S. Venkatesh
Michal Levo
Advisor: Thomas Gregor

Overlapping patterns of activity of different enhancers have been characterized through quantitative live imaging of mRNA transcription in the Drosophila embryo. Specifically, the contribution of a 'primary' and a 'shadow' enhancer to the regulation of patterning genes has been assayed quantitatively by extracting the average transcriptional activity across multiple embryos, at different developmental times and spatial coordinates, in the presence of both or only one of the enhancers. However, this previous analysis ignored much of the information on the transcriptional activity at the level of the individual nuclei, partly due to constraints in manual processing and outlier detection on the large quantities of data produced by imaging. An improved pipeline has now been developed that takes in separate stacks produced from imaging nuclei and transcription spots with different markers. The pipeline identifies nuclei and spots with minimal training, extracts spot intensity representative of transcriptional activity, and produces a data-structure and visual representation of spots assigned to their respective nuclei. The entire process has been automated with little input needed from the user, as well as optimized for batch processing so that multiple movies (tested on 65) can be analyzed together by sending jobs to a cluster. The ability to automate and batch-process data obtained from imaging has several implications for further detailed analysis. Most notably, it provides both speed and uniform processing (avoiding any manual tweaking of individual frames or movies) thereby facilitating the comparison of nuclei across time and space, and between embryos. This analysis already provides some hints to fluctuations in the transcriptional activity in single nuclei, possibly related to a phenomenon referred to as 'bursting' and to on-off switching in mRNA production. Further study into this might reveal that measures of average intensity used previously to quantify transcription patterns might obscure important underlying dynamic features.

This research was made possible by the generous support of the Lewis-Sigler Institute for Integrative Genomics.

Characterization of USP20's function as SLUG-targeting deubiquitinase

Junkai Wang
Wenyang Li, Wei Zhuo
Advisor: Yibin Kang

Epithelial-mesenchymal transition (EMT) is a reversible and dynamic process that is thought to be a crucial initiating step of cancer metastasis and invasion. During EMT, epithelial cells lose their cell polarity and cell-cell adhesion and gain migratory and invasive properties to become mesenchymal cells. SLUG/SNAI2, a zinc-finger transcription factor, transcriptionally represses E-cadherin expression, thereby promoting the EMT process. It has been reported that high expression of SLUG in various types of cancer patients is correlated with increased metastasis and relapse as well as shorter survival. Under physiological conditions, SLUG protein level is regulated by ubiquitin-mediated proteosomal degradation, but specific mechanisms still remain unclear. Previously we used siRNA screening to identify candidate deubiquitinases (DUBs) that regulate the expression level of SLUG. One of the promising candidates, ubiquitin specific peptidase 20 (USP20), significantly contributes to the stabilization of SLUG. Here, we tried to explore whether transient USP20 overexpression could decrease the ubiquitination of SLUG, thus stabilizing SLUG. Meanwhile we generated the stable cell lines which overexpress USP20 and catalytically inactive USP20 mutant (USP20 C154S), in order to investigate the role of USP20 in promoting mesenchymal characteristics and migratory ability. Further research about USP20's function in metastasis as a SLUG-targeting deubiquitinase could provide us novel insights about the regulation of EMT and cancer metastasis and establish USP20 as a potential therapeutic target.

This research was made possible by the generous support of the Department of Molecular Biology.

Molecular Characterization of Metadherin in Breast Cancer Metastasis

Nicole Wang
Heath Smith
Advisor: Yibin Kang

Breast cancer is the most commonly diagnosed cancer in women. More than 90% of breast cancer deaths are attributed to metastasis. Metadherin (MTDH), a gene that is amplified at the 8q22 genomic gain, is associated with worse prognosis of breast cancer, including increased likelihood of metastasis. While previous studies have identified MTDH's significance in promoting tumor initiation, the mechanism by which MTDH supports metastasis remains elusive. Studies in immunocompetent mice have shown that MMTV-PyMT MTDH+ cells readily metastasize to the lungs, whereas MTDH knockout (KO) cells do not significantly metastasize. However, the metastatic phenotype of MTDH KO cells is restored in immunocompromised mice. Furthermore, more regulatory T-cells infiltrate MTDH+ primary tumors than MTDH KO primary tumors. Given these findings, we hypothesize that *MTDH* expression allows tumor cells to evade immune recognition and destruction during metastasis. We predict that MTDH achieves this by recruiting regulatory T-cells to create an immunosuppressive environment favorable for cancer cell survival. To test our hypothesis, we generated an ovalbumin expression system that ensures specific interaction between tumor cells and T-cells. Through *in vitro* and *in vivo* experiments, our current results show that MTDH's immunoregulatory effects may be mediated through reduced levels of the CXCL9 cytokine. Findings from this study could result in novel immunotherapy treatments for breast cancer patients.

This research was made possible by the generous support of the Hickok Molecular Biology Senior Thesis Fund.

Regulation of the Pentose Phosphate Pathway in Cancer Cells

Monica Wei
Lukas Tanner
Advisor: Joshua Rabinowitz

A hallmark of cancer metabolism is the increase in glycolysis known as the Warburg effect. This phenomenon does not occur in isolation, however; the pentose phosphate pathway (PPP) draws from glycolysis to provide reducing power and ribose-5-phosphate that are critical for cancer cell survival. As with glycolysis, PPP activity is increased in tumor cells, but how enzymes within the PPP regulate these changes is still obscure. Better understanding of PPP regulation will help identify therapeutic targets within the PPP, which to date remain elusive. Here we study PPP regulation by overexpressing enzymes within the PPP and glycolysis. This allows us to examine the contribution of individual reactions to global pathway changes and identify reactions with significant control of pathway utilization. We used a combination of metabolomics and isotope-tracer studies to track metabolism. These techniques provide a quantitative and comprehensive analysis of an array of PPP states. We found that the PPP and glycolysis are closely regulated, as shown by broad changes in metabolite concentrations, but that most PPP enzymes do not control glycolytic flux. We investigated a particularly striking change in NADPH and NADP levels using kinetic modeling. We also observed that transaldolase overexpression led to significant changes in PPP flux. In future studies we will utilize radioactive and deuterium-labeled glucose tracers to further estimate PPP flux.

This research was made possible by the generous support of the Lewis-Sigler Institute for Integrative Genomics.

Effects of Overactivating MEK Mutations in ERK Signaling and *Drosophila* Morphogenesis

Kei Yamaya
Yogesh Goyal, Granton A. Jindal, Rebecca Burdine, Trudi Schüpbach
Advisor: Stanislav Y. Shvartsman

The Ras/ERK signaling pathway is implicated during multiple stages of embryogenesis and for adult homeostasis. The ERK signaling pathway is activated in response to extracellular signals that activate the receptor tyrosine kinase (RTK). ERK activation is tightly controlled both spatially and temporally, and ectopic activation of the pathway during embryogenesis leads to a class of developmental disorders known as the RASopathies. In particular, MEK, a kinase directly upstream of the effector molecule ERK, can be mutated in a number of domains and can lead to various facial and structural defects, such as Cardio-facio-cutaneous (CFC) syndrome, and cancer.

In this study, we focused on the effects of overactivating MEK mutations on ERK signaling and on embryonic morphology. Surprisingly, we found that these gain-of-function mutations do not uniformly elevate ERK signaling levels, but that they decrease ERK signaling levels at the poles of the embryo. This ectopic activation and attenuation affects expression of genes downstream of the pathway. For example, the posterior expression domain of the gap genes *tailless* and *hunchback* were expanded by two to three-fold in these mutants. Finally, ectopic ERK activation leads to opposing effects in morphology of the embryo. While reduced and fused abdominal segments were observed in the the middle of the mutant embryos, loss of anterior structures was also observed in these mutants. This abnormal terminal structure phenotype is similar to that observed in loss-of-function MEK mutants. These results indicate that the biological effects of activating mutations in embryos are likely to reflect a combination of ectopic pathway activation and altered responses to endogenous cues.

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Analysis and Comparison of Nuclear-Cytoplasmic Ratio at the Mid-blastula Transition in *Drosophila* species

Karen Zhang
Katherine Angier
Advisor: Amanda Amodeo

In *Drosophila melanogaster* embryos, the developmental stage known as the mid-blastula transition (MBT) has been found to be regulated in part by the nuclear-cytoplasmic ratio (NCR). The MBT is a significant developmental milestone for *Drosophila* embryos because it is at this stage that major changes including activation of zygotic transcription, transformation of chromatin architecture, and restructuring of the cell cycle occur. However, the regulation and timing of these changes are not well-studied in other *Drosophila* species. We measured the nuclear-cytoplasmic ratio at MBT of six *Drosophila* species: *D. busckii, D. mauritiana, D. melanogaster, D. mojavensis, D. persimilis,* and *D. virilis*, to investigate possible factors that affect the NCR, such as egg size or developmental timing. In calculating the NCR, it was necessary to find the average embryonic volume for each species. One interesting result we found from these measurements was that, contrary to previously published information, *D. virilis* embryos are not significantly larger than *D. melanogaster embryos*, but are in fact slightly smaller. The other metric needed to calculate NCR is the total number of nuclei in an embryo at MBT. From this data, we concluded that all species entered MBT after 14 cell division cycles. Furthermore, we found that the total number of nuclei did not scale with embryonic volume, but rather stayed mostly constant across all species. We also investigated a slightly different metric, the ratio of DNA content to cytoplasm (DNACR), by taking the genome sizes of the various species into account, and found that there are significant differences in DNACR between different species.

This research was made possible by the generous support of the Lewis-Sigler Institute for Integrative Genomics.

The Regulatory Role of Hypodermis Genes in Fat & Glycogen Metabolism in *C. elegans*

Qianyun Zhang
Rachel Kaletsky, Cheng Shi
Advisor: Coleen T. Murphy

Caenorhabditis elegans is a widely-used model organism in aging studies. Hypodermis is a skin-like tissue in *C. elegans*, which has many different functions, such as nutrient storage and cuticle secretion. However, much remains to be understood about this unique tissue and its role in aging. Our lab has developed a novel technique that uses fluorescence-activated cell sorting (FACS) to specifically isolate hypodermis cells from adult worms while closely maintaining the *in vivo* transcription profile. After isolating the hypodermis cells, RNA-Seq analysis was performed to identify genes that are important in hypodermis function. Based on this method, 30 hypodermis-enriched genes with potential fat and glycogen metabolism function were selected, and it is hypothesized that these genes may affect fat and glycogen metabolism, and possibly aging, through activity in the hypodermis. To test this, RNAi was used to knockdown candidate genes and then perform Oil Red O Staining and Iodine Glycogen Staining to measure the total worm fat and glycogen content, respectively. Preliminary results suggest that knockdown of these genes may affect total worm fat and glycogen. However, high-throughput analysis software is still needed, and ongoing studies need to repeat our preliminary results. These results suggest that hypoermis-expressed genes in adult animals play a previously unappreciated role in fat and glycogen metabolism that may be important in the regulation of aging.

This research was made possible by the generous support of the Molecular Biology Department and Global Health Program.

A Screen for Residues Involved in Pseudomonas aeruginosa Quorum Sensing Inhibition by Flavonoid Compounds

Fangzhu Zhao
Amelia R. McCready, Jon Paczkowski
Advisor: Bonnie L. Bassler

Quorum sensing is a form of bacterial cell-cell communication used to control collective behaviors including virulence and the formation of biofilms. The opportunistic pathogen *Pseudomonas aeruginosa* possesses two main quorum-sensing systems LasI/LasR and Rhll/ RhlR. Our lab has found that flavonoid compounds can inhibit quorum sensing in *Pseudomonas aeruginosa*, but the mechanism is unknown. In this study, we generated a RhlR random mutant library to screen for residues involved in quorum sensing inhibition by flavonoid compounds. We also used site directed mutagenesis to generate additional mutations in the putative binding pockets of LasR and RhlR. Our results indicate that, Ser129 and Tyr56 in LasR mutants are crucial residues for binding with quorum sensing inhibitors. We found that S129F and Y56R strongly respond to flavonoid compounds while S129C and Y56F are dramatically inhibited by flavonoid com-pounds with mBTL as an agonist. Besides, mutant versions of RhlR receptor reveal that Y64F is resistant to flavonoid compounds. This work helps us to narrow down where flavonoid compounds bind and how they interact with LasR and RhlR.

This research was made possible by the generous support of the Howard Hughes Medical Institute.

Mating Induces Fat Loss in *Caenorhabditis* Hermaphrodites

Shiyi Zhou
Cheng Shi
Advisor: Coleen T. Murphy

It has been known that reproduction affects storage and metabolism of fat in animals. Previous studies provide evidence that reduced reproduction leads to the increase of fat storage in many species. More studies revealed that ablation of germline significantly alters lipid metabolism. However, the mechanism remains poorly understood. Here we discovered that mating with males induces fat loss in wild type *Caenorhabditis* Hermaphrodites. However, surprisingly we found that without having germline, hermophrodites also lose fat in their bodies after mating. Also, in wild type *Caenorhabditis* mothers, the fat lost in germline is dramatically less than that in somatic cells, which indicates there may be redistribution of lipid from intestine to germline. We also found out that the fat storage in the worms mated with males that can't transfer sperm is almost the same with that of *Caenorhabditis* mothers mated with normal males, which indicates that it may be sperm that causes reduction of fat storage in *Caenorhabditis* Hermaphrodites. In the future we will do microarray to find some genes that may be responsible for fat loss after mating. Our study provides insight to the mechanism how mating generates the reduction of lipid storage in *Caenorhabditis* Hermaphrodites, which may be an explanation of how reproduction influence fat metabolism in many species. Also, our experiments tried to uncover the molecular mechanisms that regulate lipid metabolism after mating.

This research was made possible by the generous support of the Department of Molecular Biology.