Pseudorabies Virus Induces Interleukin-6 Specific Immune Response in Mice
Kristen Albrecht
Kathlyn Laval
Advisor: Lynn Enquist

Pseudorabies virus (PRV), a neurotropic alphaherpesvirus, establishes asymptomatic infection in swine. In non-native hosts, including mice, PRV causes mad itch and death. Studies with a flank inoculation model found that mice infected with PRV develop pruritus in the flank by 40 hours post-inoculation and die approximately 72 h. Pruritus does not cause death, as has been shown by a study observing the same time of death for anesthetized and non-anesthetized mice. We hypothesize that death results from a lethal uncontrolled immune response initiated by PRV replication in the peripheral nervous system and spread to the spinal cord. This study aims to characterize the currently undefined immune response. Mice were inoculated in the hind footpad, blood was collected every 24 hours, and tissues were collected from animals sacrificed at 72 h. Among 11 pro-inflammatory cytokines, only IL-6 was significantly increased in sera of infected animals at 72 h. Immunohistochemistry staining detected PRV in the foot, bladder, kidney, heart, and sacral through thoracic segments of the spinal cord but not in the cervical spine, brain, lungs, liver, spleen, or pancreas. Cells producing IL-6 were detected only in the inoculated foot and heart. Therefore, we conclude that PRV spreads from the inoculation site to the spinal cord through peripheral neurons, and replication initiates an IL-6 specific inflammatory response. A time course study of IL-6 secretion in these tissues collected every 12 hours will be performed using ELISA and q-PCR to establish whether secretion correlates with the development of clinical signs, particularly pruritus.

Probing the role of cyclophilin A in hepatitis C virus (HCV) replication and host tropism
Metodi Balev
Jenna M. Gaska, Brigitte Heller
Advisor: Alexander Ploss

Hepatitis C virus (HCV) is a leading cause of liver-related mortality. Although tissue culture systems allow for the study of HCV in vitro, in vivo studies have been restricted by the poorly understood host tropism of the virus and lack of an easily-accessible, externally-valid animal model. Mice genetically engineered to express critical human HCV entry factors are permissive to infection, but viral replication remains low. One potential barrier could be the incompatibility of murine orthologs of vital host factors with components of the HCV replication machinery. Recent evidence suggests that even minor deviations in the primary sequence of the peptidylprolyl isomerase cyclophilin A (CypA), a host factor crucial for HCV replication, can have noticeable effects on HCV replication efficiency in human-derived hepatocytes. In this study, we seek to explore the contribution of interspecies deviations in CypA protein sequence to both the restricted host tropism of HCV and the poor replication efficiency of HCV in murine cells. We utilized human hepatoma cells expressing an shRNA directed against endogenous CypA (Huh7.5-shRNA CypA) transduced with CypA orthologs from a variety of species and assessed these orthologs’ respective ability to rescue HCV infection. Given our preliminary findings that murine CypA (mCypA) cannot facilitate efficient viral replication and conversion of certain mCypA residues to the analogous human sequence confers an increased ability to promote viral replication, we will perform AP-MS on FLAG-tagged human CypA (hCypA) and mCypA to determine the differential protein interactions during HCV infection that may affect mCypA’s inability to promote viral replication.
An epistatic basis for the correlated clustering of amino acid substitutions

Logan Blaine
Andrew Taverner
Advisor: Peter Andolfatto

The extent to which protein evolution is predictable remains an important open question in evolutionary biology, necessitating a better understanding of the forces of evolutionary constraint. Previous work by our lab has shown that amino acid substitutions, sites within a protein that differ between species, tend to cluster within the DNA coding sequence (CDS) of a protein. This clustering may in part be explained by epistasis, whereby a prior substitution at a given site in a protein influences the effect that substitutions at neighboring sites have on the protein’s function. An in silico model of purifying selection on a set of proteins was built to test whether epistasis between substitutions is sufficient to cause clustering. In this simulation, substitutions are assigned a higher fitness if they do not substantially effect the protein’s free folding energy, as calculated by the software FoldX. Preliminary results from the simulation reveal that sequential substitutions occur slightly closer together under our model than those introduced alone, implicating epistasis as causative force for clustering. However, the magnitude of this clustering is far lower than the clustering observed in actual genomic data, suggesting that positive selection, rather than purifying selection, may produce most of the observed clustering. To complement the in silico study, I also have begun a computational analysis of Drosophila genomes to identify clusters of substitutions that cannot be explained by site-specific differences in evolutionary constraint. Through this, it will be possible to quantify the prevalence of epistatic clusters of substitutions across the genome.

Investigating the Role of the Planar Cell Polarity Pathway in Mammalian Epidermal Wound Repair

Sandra Carpenter
Sara Stahley
Advisor: Danelle Devenport

Efficient repair of wounds to the skin is a fundamental capability of an organism’s survival. Achieved by migrating keratinocytes, reepithelialization is an inherently polarized process involving coordinated and directional collective cell movements. These highly orchestrated movements remain incompletely understood. Recent work indicates a potential link between the Planar Cell Polarity (PCP) pathway and a murine cytoskeletal effector necessary for epidermal repair. PCP regulates a diverse range of embryonic morphogenetic processes that are recapitulated in wound closure. It is conceivable that the wounded state triggers upstream polarity cues that then exert an influence on the complex coordination of collective cell movements during healing. To define a more precise role for PCP in wound repair, this research seeks to learn how epithelial polarity changes at the molecular level during and after wounding as well as to describe how disrupted PCP affects cell behavior. Using in vitro and in vivo wounding assays on murine skin, this work will specifically characterize patterns of protein localization in response to wounding, predicting that PCP components will reorient at the wound margin to demarcate leader cells from more distant follower cells. The results from these experiments may provide direction for the future study of cell polarity as an informer and regulator of healing with emphasis on dissecting wound-specific associations between PCP proteins and their downstream cytoskeleton effectors. A better understanding of the molecular mechanisms underlying wound repair is valuable for medical advances in surgery, tissue repair and regeneration, and certain cancer pathways.
The temporal dynamics of dopamine in working memory
Jessica Chambers
Advisor: Ilana Witten

Working memory, the basis of learning and cognition, is a system for temporary storage and management of novel information that directs subsequent behavior. Several lines of research suggest that dopamine plays a crucial role in working memory function and may improve its capacity. However, the specific aspects of working memory that the dopaminergic system support have yet to be fully explored, as previous experimental approaches have been hindered by a lack of temporal resolution and cell-type specificity. Optogenetics presents a promising solution to these difficulties, as it enables direct manipulation of neuronal activity at a rapid timescale that matches dopaminergic neuronal activity. Our unpublished preliminary data shows that SNc dopaminergic neurons are causal to working memory, as optogenetic inhibition of these neurons throughout a working memory task impairs performance accuracy. These results motivate my project: focused investigation of neuronal activity during specific epochs of the task, each of which maps to a phase of working memory. The experiments proposed here will unveil fascinating and central aspects of behavior, while furthering our understanding of related disease processes. Furthermore, to our knowledge, these experiments are among the first to link temporal dynamics of the dopaminergic system to the regulation of working memory.

ARDS is Associated with Genome Wide Methylation Changes and Alterations in DNA Methylation Age in Adults
Sam Chiacchia
Lisa Schneper, Iulia Kotenko, Gaby Soto
Advisor: Daniel Notterman

Acute respiratory distress syndrome is a lethal condition of acute bilateral lung disease associated with trauma, sepsis, and shock that occurs as a result of fluid build up in the alveoli. Prior work in the field suggests that ARDS and its pathophysiology may be mediated by epigenetically controlled factors, such as DNA methylation, and that the syndrome itself may incur genome wide DNA methylation changes. In this investigation, we compare DNA methylation data derived from blood collected from 39 adults with ARDS, 75 ICU controls, and 30 healthy individuals made available by Szilagyi et al. Probe wise analysis revealed genome wide methylation changes across 24 chromosomes in the promoters of 13,475 genes, while regional analysis of differential methylation revealed 67 significant regions across 21 chromosomes. GO analysis revealed significant enrichment for CpG sites associated with response to wounding and stress. Taken together, these results demonstrate that the human methylome is significantly altered in response to ARDS, suggesting a potential role in epigenetic response to the stress of acute lung injury.
Antimicrobial-Encoding Biosynthetic Gene Clusters from *Lactobacillus* Strains in the Vaginal Microbiome Mediate Bacterial Community Dynamics

Diana Chin  
Jared Balaich  
Advisor: Mohamed Donia

The lactic acid-producing genus *Lactobacillus* dominates in vaginal bacterial communities in healthy women and is thought to play a key role in protecting against pathogens. Consistent with this idea, bacterial vaginosis – involving reduced proportions of vaginal *Lactobacillus* species – is correlated with adverse health outcomes such as preterm birth and HIV transmission. Specific *Lactobacillus* species within the vaginal microbiome are associated with different outcomes, indicating factors besides lactic acid that influence host-pathogen response. The mechanisms behind the community dynamics of the vaginal microbiome remain poorly characterized. We hypothesized that strains of *Lactobacilli* may secrete antimicrobial small molecules that confer a competitive advantage over both commensals and pathogens. To investigate, we queried the genomes of 38 *Lactobacillus* strains for antibiotic-encoding gene clusters found in bacteria and identified sixteen distinct biosynthetic gene clusters (BGCs). Using a twofold approach, we cloned candidate BGCs and expressed them heterologously in *Bacillus subtilis* and also expressed them endogenously in the native *Lactobacillus* strains. We tested both types of cell extracts for activity against a panel of pathogens and found evidence for activity against Gram-negative bacteria from two *L. jensenii* strains carrying different BGCs. We are currently testing additional strains using disc diffusion bioassays. Together, our preliminary results suggest that antimicrobials encoded by BGCs found in certain *Lactobacillus* strains could play an important role in mediating the dynamics of the vaginal bacterial community by inhibiting certain pathogens.

Phenotypic and Genetic Analyses of 2D61, a Unique Embryonic Lethal Mutation in *Drosophila Melanogaster* (in the Wingless pathway)

Mary Kate Davis  
Advisor: Eric Wieschaus

*Drosophila melanogaster* develop utilizing a set of signaling pathways that are highly conserved in most species. The basic structure of these pathways is well understood, but their regulation during development is not known. The 2D61 mutant stock produces abnormal embryos with a phenotype resembling a hypomorphic mutation in the wnt/wingless pathway. Understanding the genetic basis of this stock could provide new insight into the modulation of these pathways. I compared the cuticle from 2D61 with known developmental mutants in various signaling pathways. 2D61 has similar denticle belt malformations as a hypomorphic armadillo/betacatenin mutant. My preliminary analyses using classical genetic mapping shows that there are two lethal mutations in 2D61, localized at 2-57 and 2-64. The first lethal mutation causes both the denticle belt and head defects, while the second lethal mutation shows a normal phenotype. Because there are no known components of the wingless pathway in this area of the genome, a novel gene is likely perturbed by this mutation. Another mutation is available from the nation stock center, 3263, that is reported to show a similar phenotype but in my analysis complements 2D61. Their head phenotypes are similar, but the denticle belt malformations in 3263 are similar to an arrow mutant, another component of the wingless pathway. The two lethal mutations were isolated and along with 2D61 will be tested further by utilizing scanning electron microscopy for brown head defect analysis, movies for temporal developmental divergence, and genetic localizations using deletion and deficiency complementation testing. Ultimately this project will determine the role of this novel gene in the regulation of such a highly conserved pathway.
Investigating Translation Inhibition by RNase L
Kaitlin Demarest
Sneha Rath
Advisor: Alexei Korennykh

In response to cell stress (more specifically, double-stranded RNA), the endoribonuclease RNase L is activated. When active, RNase L cleaves noncoding RNAs and inhibits translation, although the complete mechanism of this inhibition has yet to be determined. By measuring translation activity after activation of wild type and cleavage-deficient RNase L, we have demonstrated that its cleavage ability is necessary for translation block. We have also shown that RNase L is capable of blocking translation in *Escherichia coli*. Thus, we hypothesize that RNase L is targeting a part of translation conserved in humans and bacteria. We aim to deepen our understanding of RNase L’s method of inhibition in a number of ways. First, we plan to study its effect on tRNA in bacteria by analyzing their cleavage and acylation status. Second, in order for the stress response to be successfully carried out, we believe that certain transcripts must be able to evade this block. We hope to identify these transcripts, as this could clarify how the block is evaded and provide insight into how RNase L establishes it. To do this, we plan to use puromycin immunoprecipitation to isolate nascent polypeptides and identify them via mass spectrometry. These findings will expand our knowledge of the cell in times of stress, and could ultimately lead to the development of cancer therapies that target translation, as there are a growing number of anticancer drugs that shut down protein synthesis.

Investigating the Relationship between Metabolic Activity and Persistence in Growth-Inhibited Populations of *Escherichia coli*
Nazik M. Elmekki
Grant M. Gelderman
Advisor: Mark P. Brynildsen

Resistance to antibiotic therapies is an increasing global health problem, and nonheritable bacterial persistence poses an independent set of challenges.Persisters are bacterial cells that can tolerate antibiotic treatment independent of resistance genes and naturally comprise a small fraction of bacterial populations. These persistent cells are thought to be responsible for relapse infections, with nongrowing populations being particularly difficult to kill. There have previously been several investigations into persister metabolism, but with a focus on metabolic activity prior to antibiotic treatment. For this study, we used the fluorescent dye Redox Sensor Green (RSG) to characterize the metabolic activity of stationary phase *Escherichia coli* populations during ofloxacin treatment, followed by fluorescence-activated cell sorting (FACS) to assess the dependence of persistence on metabolic activity during treatment. To survive ofloxacin treatment, DNA repair machinery must be active in persisters, and thus we hypothesize that persisters require higher rates of cellular metabolism. Preliminary experiments have established that RSG does not affect the ability of ofloxacin to kill stationary phase cells and that an RSG signal accumulates over the course of a 6 hour incubation following antibiotic treatment. The findings of this study will provide insight to persister cellular metabolism and their response to antibiotic stress, paving the way for the development of new drug therapies that are more effective against persistent populations. Future studies may investigate the metabolic response of persisters when exposed to additional antibiotics, explore different nutrient and environment conditions, or more closely examine the metabolic processes during treatment that enable survival.
Conserved Neuronal Firing Sequences in the Hippocampus: An Application of Graph Theory’s the Longest Paths Problem

Zach Feig
Edward Nieh
Advisor: Carlos Brody

The mouse hippocampus is an important brain region implicated in navigation, decision-making and reward seeking behavior. Activity in this region can be measured via two-photon calcium imaging. Recent advances in the imaging techniques, surgical procedures, mouse restraint and virtual reality allow both wider field and higher resolution hippocampal imaging while the mouse completes behaviors. These advances allow us to resolve 10 times as many Regions of Interest (ROIs) as previous comparable studies, however, the ability to analyze this new data lags behind our capability to collect it. Stereotyped patterns of neuronal activation are notoriously hard to find in hippocampal brain data. Hippocampal data cannot be generalized between subjects, or between imaging sessions because this region of the brain has a tendency to remap. Even in a single recording session, complete consensus sequences are unlikely to be found in single trials because ROIs are activated probabilistically.

This problem can be reduced to the graph theory problem longest paths. In order to identify long sequences we composed a graph consisting of all ordered pairs (doublets) of ROIs occurring in all trials. Edge weights were equal to the number of trials in which the doublet appeared and the directionality was the activation. This graph should contain long, weighty, and acyclic paths – in other words, viable consensus sequences. Unfortunately the longest paths problem is NP-hard, meaning it cannot be solved in polynomial time with any known algorithm. We suggest several case specific heuristics that make this problem more manageable and allow discovery of possible paths.

Nephronectin and Tenascin-C may be key players in mesangial matrix expansion in diabetic nephropathy

John B. Finlay
Maria E. Vega
Advisor: Jean E. Schwarzbauer

Diabetic nephropathy (DN) is a leading cause of end-stage renal disease and results from an overproduction of extracellular matrix (ECM) in the glomeruli of the kidneys where filtration occurs. Our lab has shown that mesangial cells, which normally provide support to surrounding tissue in the glomeruli, increase fibronectin matrix assembly when grown in 30mM versus 5mM glucose. However, the precise molecular mechanisms of gene and pathway regulation that contribute to ECM over-assembly remain unclear. We performed RNA-Seq on mesangial cell cultures grown in 5mM or 30mM glucose for 72 hours to determine high glucose induced changes in gene expression. DESeq2 and KEGG pathway analysis revealed induction of the bone morphogenetic protein 2 (BMP2) signaling axis and significant upregulation of the potentially downstream kidney developmental protein nephronectin (NPNT). Interestingly, NPNT has recently been suggested as a reliable marker for DN, but its role in the adult kidney is unknown. Immunoblotting revealed that NPNT localizes to the medium as a secreted protein, and time course quantitative-PCR, consistent with our hypothesis of gene regulation, showed that BMP2 expression increases before NPNT. Furthermore, the ECM glyco-protein Tenascin-C (TNC) was significantly upregulated in high glucose, which we show is sufficient to cause alternative splicing of TNC. These results indicate that ECM over-accumulation in high glucose conditions may be mediated specifically by NPNT and TNC.
Analysis of Post-Initiation Translational Regulation during Drosophila Oogenesis by Ribosome Footprinting

Zachary Flamholz
Yingshi Peng
Advisor: Elizabeth R. Gavis

Post-transcriptional gene regulation is increasingly being recognized for its role in spatial and temporal control of protein expression. Research of one such mechanism, translational regulation, has traditionally focused on regulation of the pre-initiation and initiation stages of translation. However, there are situations that necessitate the ability to quickly switch between translation and repression. Such ability could be conferred by a post-initiation regulation mechanism, where ribosomes are paused in the process of elongation and can resume translating upon removal of the pausing mechanism. Early Drosophila oogenesis presents a good model for studying such a mechanism, as many maternal mRNAs dumped from the nurse cells into the oocyte require immediate translational repression. Additionally, previous biochemical work in our lab suggests that nanos is regulated at the post-initiation step. Ribosome footprinting allows for mapping the position of translating ribosomes with codon level resolution. Using previously collected footprint data as well as data published by the Bartel lab at MIT, a pipeline was developed for identifying possible ribosome stalling sites, manifesting as peaks in fragment coverage within a gene coding region. Five peak containing genes were discovered across three independent wild-type Drosophila late-stage oocyte footprint samples. Three of the peaks are maintained in footprint data from three early embryo datasets. Additional footprint samples will be used to confirm peaks and identify others. The results suggest the prevalence of an elongation based translational regulation mechanism. Further research will focus on characterizing the distribution of peaks as well as elucidating the mechanism of peak formation.

Neuronal Feature Encoding in the Hippocampus CA1 Region during an Evidence Accumulation Task in Mice

Nicolas Freeman
Edward Nieh
Advisor: Carlos Brody

The hippocampus has been previously described to encode spatial information as a ‘cognitive map’ of the current environment. Although individual cells, including place cells, have been shown to encode certain features of the environment, further research is required to more thoroughly classify and understand how these cell types are linked to behavior. In this study, we have utilized two-photon microscopy to measure large-scale neuronal activity in the hippocampus CA1 of Mus musculus during an evidence accumulation task. For the evidence accumulation task, a head-restrained mouse runs along a virtual reality T-maze during which visual cues appear on the left or right side; at the end of the maze, the mouse decides to turn left or right, the side with the greater number of cues indicating the correct choice. By recording several features of the task, including cue position, head angle, and task difficulty, we can analyze activation events of single neurons along multiple feature dimensions. First, one-dimensional feature analyses of a neuron’s probability density function suggest which features a neuron may encode. Multidimensional k-means clustering analyses may be used to determine how the features a neuron encodes interact with each other during the behavioral task. Future steps include neuron classification across the population based on feature encoding. Validity of the classification analyses will be analyzed with resampling shuffle tests.
Characterizing Human RASopathy Mutations in *Danio Rerio*

Noah Han
Victoria Patterson
Advisor: Rebecca Burdine

RASopathies are diseases that arise from germline mutations in the RAS/MAPK signaling pathway and affect ~ 1 in 1000 live births. Patients with RASopathies display symptoms including congenital heart disease, short stature, craniofacial defects, and neurocognitive delay. One of the most common RASopathies is Noonan syndrome (NS), affecting 1 in 2000 births, displays typical RASopathy symptoms as well as an increased risk of developing cancer. Half of all Noonan syndrome cases arise from mutations in Src homology phosphatase 2 (SHP2). Mutations that cause an increase in SHP2 activity lead to the upregulation of RAS/MAPK signaling leading NS, while mutations inactivating SHP2 cause Noonan Syndrome with multiple lentigines (NSML). In this study, I will rank mutant SHP2 variants for their impact on development events of *danio rerio* (also known as zebrafish) by microinjecting site-directed mutagenized mRNA into the 1-cell stage embryo. Since RASopathies in humans appear as heterozygous polymorphisms I will generate RASopathies patient mutations in *ptpn11a* (the gene encoding Shp2) through CRISPR/Cas9.

Functional annotation of gene set enrichment analysis generated networks gives an identity to significantly modified pathways and functions

Gabriel Joseph
Adam Oberstein
Advisor: Tom Shenk

Gene set enrichment analysis reduces the redundancy in differential expression assays, which measure changes in gene expression levels. By functionally annotating communities of gene sets that are concordantly regulated, we can name and identify processes and functions that are significantly modified under experimental conditions. This analysis, while computational in nature, allows us to sift through large amounts of data and understand mechanistically how viruses (or any family of perturbation) effect endogenous cell function. We set out to determine whether it would be possible to look at a community of related gene sets, and automate a system that uses their identities and naming conventions to characterize the family with one term. Our method involves climbing up a clustering tree where members of a community that are closest together, are most closely connected on a tree – the x-y coordinates of a graphical representation of the gene set network, define proximities. Our system allows us to input gene set expression data, pass it into a Javascript that generates a force directed graph, and process it once again in R to name communities. This tool has incredible potential, as you can very quickly identify which cellular functions are significantly modified across different viruses, different time points, all through a pipeline that allows for immediate and side-by-side comparisons.
By converting sunlight into organic compounds, photosynthesis produces essential biomatter necessary for the maintenance of life on Earth. However, photosynthetic processes such as carbon concentrating mechanisms (CCM) remain relatively undefined. Research into the genetic underpinnings of photosynthetic unknowns can be conducted in model organisms like *Chlamydomonas reinhardtii* which is ideal for the genetic study because of its 1) well characterized genome 2) known reproductive patterns 3) significant comparability to higher plants. Insertional mutagenesis of known DNA cassettes into the *Chlamydomonas* genome has been used to generate a photosynthetic mutant *Chlamydomonas* library. This study aims to examine 264 previously uncovered low confidence candidate genes with potential correlation to *Chlamydomonas* photosynthesis. The progeny arising from test crosses between mutants of interest and WT strains will be phenotypically screened for photosynthetic abnormalities. PCR assays will be used to verify the presence and location of the insertion within candidate genes predicted to correlate to photosynthetic processes. This work will eliminate mutants containing background secondary site mutations from the pool photosynthetic candidate genes. Additionally, relevant literature will be examined to determine if any of the 264 unannotated candidate genes have been previously implicated in photosynthetic pathways to elucidate their function and importance in *Chlamydomonas* photosynthesis.

**An Investigation of a Novel Host Determinant of Live-Attenuated Yellow Fever Virus Infection**

**David Kim**

Florian Douam, Gabriela Hrebikova, Qiang Ding

Advisor: Alexander Ploss

Yellow fever virus (YFV) is a prototypic member of the *Flavivirus* genus that continues to cause a significant global burden. A live-attenuated vaccine strain, termed YFV-17D, is available and provides long-term immunity with a single dose. Despite the vaccine’s high efficacy and a mere 32 amino acid variations from its virulent parental strain, YFV-Asibi, the mechanism of viral attenuation and the specific determinants governing the virulence of the YFV strain remain poorly understood. Data from our lab support the notion that the effective viral replication process of YFV-17D is specific to primate hosts, and therefore specific host components in humans may contribute to the efficacy of the YFV-17D. However, no such host components, to our knowledge, have currently been identified. Recently, the Ploss lab performed a genome-scale CRISPR-Cas9 functional screen on hepatoma cell lines to identify such host factors essential for YFV-17D replication. We isolated a cell clone exhibiting a strong resistance to YFV-17D infection. This resistance phenotype was associated with a disruption in a gene locus coding for a protein – tentatively referred to as S23 – involved in cell signaling. Preliminary experiments indicated that this protein might play a role on viral RNA replication and viral particle release. In depth characterization of this protein’s involvement in the replication cycle of YFV-17D, along with the identification of human-specific determinants, could provide novel insights into the immunogenicity mechanisms of YFV-17D. This research could then potentially help develop novel vaccine strategies for other members of *Flavivirus* family such as Zika, Dengue, and West Nile viruses.
Photoswitchable Control of Protein-Protein Interactions: Engineering Light-Sensitive DARPins

Joshua Kim
Agnieszka Gil
Advisor: Jared Toettcher

The recently developed technology of optogenetics utilizes photoactive proteins to enable control over intracellular processes. One of the well-studied families of photoactive proteins is the Light, Oxygen, Voltage (LOV) domain family, including the Avena Sativa LOV2 (AsLOV2) protein that has been used to create a toolbox of photoactivatable signaling systems in vitro and in vivo. Recently it was reported that by inserting AsLOV2 into a solvent-exposed loop of a protein of interest, it is possible to destabilize a protein upon light stimulation, reversibly. However, this technique requires a solved crystal structure of the protein of interest along with extensive computational work to correctly insert the AsLOV2 domain. Here, we propose a novel approach based insertion of the LOV domain to construct a library with insertions at all possible positions. We will then screen for photoswitchable binding to a native target of the protein of interest. We will test the viability of this approach in the context of two ERK2-specific DARPins, E40 and pE59. DARPins represent a large class of engineered proteins that all utilize a highly conserved epitopes, therefore we expect the photoswitchable binders we identify to be highly generalizable and easily translated to other classes of DARPins. If successful, our approach will demonstrate the viability of a transposase insertion approach to insert LOV domains into any protein of interest to implement light-controlled binding interactions with the benefit of engineering new optogenetic tools without prior knowledge of the protein’s structure. We have already shown that AsLOV2-DARPin fusion proteins can be expressed and purified, with functional and photoswitchable AsLOV2. The AsLOV2-DARPins will be tested for photoswitchability by using LED plates, and tested for ERK2 binding in mammalian cells using Kinase Translocation Reporters (KTR) assays.

Hysteresis control of TGFβ-induced epithelial-mesenchymal transition generates a distinct program with metastatic abilities

Daniel D. Liu
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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 tumor cells to disseminate through the body and establish distant site metastases. The signals and transcription factors (TFs) inducing EMT are widely known, which converge to suppress the expression of epithelial genes, most prominently E-cadherin. However, less is known about the intrinsic spatiotemporal dynamics of the transition. Mathematical modeling and experimental validation of TGFβ-induced EMT shows a non-linear response resulting in a hysteretic bistable switch in E-cadherin expression, tightly controlled by the Zeb/miR-200s double-negative feedback loop. In other words, cells are predicted to undergo all-or-nothing transitions between epithelial- and mesenchymal-like states. Using CRISPR/Cas9-mediated genome editing, we disrupted a critical interaction breaking the Zeb/miR-200s feedback loop, which successfully eliminated hysteresis in vitro. We show that hysteresis ensures quick responses and molecular memory, which sustains the EMT-like state long after withdrawal of stimuli. Remarkably, cells gained invasive properties equally regardless of the type of transition; however, only cells following hysteresis increased lung metastatic colonization. Accordingly, only cells undergoing hysteretic EMT differentially expressed subsets of stem cell-like and extracellular matrix genes with clinical prognosis value. Overall, these findings illustrate the existence of distinct EMT programs which have functional effects on metastatic properties.
Characterization of the host-virus mitochondrial interactome during HCMV infection

Clayton Otter
Joel Federspiel
Advisor: Ileana Cristea

In order to successfully infect its host, human cytomegalovirus (HCMV) must both evade the host immune response and hijack cellular machinery to manufacture and export virus progeny. Recent work by our lab and others has shown that HCMV has a profound effect on the mitochondria, as increased mitochondrial fragmentation and localization to the periphery of the virion assembly complex (AC) during infection has been observed. Our project aims to identify virus-host interactions that may be responsible for these changes in the mitochondria. In order to characterize the mitochondrial virus-host interactome, we used immunoprecipitation-mass spectrometry (IP-MS) techniques. We first optimized mitochondrial fractionation, viral protein extraction from the mitochondria, and isolation of tagged viral proteins. Then, we applied these optimized methods to examine the interactome of viral protein pUL37x1. Though the mitochondrial-localized pUL37x1 has been studied extensively for its role in preventing host cell apoptosis, no interaction network studies for this protein have been conducted. Following infection by a virus that expresses pUL37x1 tagged with GFP, mitochondria were isolated, IPs were conducted to pull-down pUL37x1 along with any interacting proteins, and MS was used to identify these interactors. These preliminary experiments have identified specific host and viral proteins as pUL37-interacting partners that play a role in various cellular pathways, including mitochondrial translation and protein processing. A better understanding of this interactome will provide further insight into the progression of infection and can help identify targets for the development of antiviral treatment.

Interferon-inducible protein 16 (IFI16) in immune signaling during herpesvirus infection

Catherina Pan
Krystal K. Lum
Advisor: Ileana M. Cristea

Human interferon-inducible protein IFI16 is a sensor of exogenous DNA critical for intrinsic and innate immunity. During herpes simplex virus-1 (HSV-1) infection, IFI16 represses viral gene transcription and induces antiviral cytokine expression to suppress pathogenesis. However, how IFI16 initiates these downstream immune responses remains unclear. Our lab and others have previously demonstrated that IFI16 co-localizes with incoming viral genomes and promyelocytic leukemia (PML) proteins, and that its pyrin domain (PYD), which functions in homotypic oligomerization, is necessary for these localizations. Since protein oligomerization occurs among several immune signaling proteins, and PML proteins are well-known for their antiviral functions, we hypothesize that the ability to oligomerize is necessary for IFI16 antiviral functions. Moreover, this study aims to determine the downstream mechanism by which IFI16 oligomerization exerts these effects. To address these questions, we created oligomerization-disrupted PYD mutants and assessed the functional consequences of non-oligomerizing IFI16 in the context of HSV-1 infection. Using immunofluorescence, western blotting, and quantitative real-time PCR, we found that these PYD mutants exhibited increased viral gene expression and protein levels when compared to wildtype IFI16, indicating that PYD oligomerization is important for IFI16 antiviral functions. With the rise of gene therapy and DNA-based vaccines, this study’s findings will lend key insight into the interaction of the immune system with herpesviruses, and may enable the development of safe and effective vaccines against viral, bacterial, and parasitic diseases.
Characterizing the role of DACT1 in Wnt signaling and breast cancer bone metastasis

Nana Park
Mark Esposito
Advisor: Yibin Kang

Metastasis, the dissemination of tumor cells to distant parts of the body, accounts for 90 percent of cancer-related fatalities, and among breast cancer patients, 70 percent of metastatic burden can be attributed to metastasis to the bone. Yet, no reliable markers exist to prognose breast cancer patients at high risk of developing bone metastasis. To identify bone metastasis promoting factors, we screened for genes over-expressed at least two-fold in highly bone metastatic derivatives of breast cancer cell lines. Among genes identified, Dishevelled Binding Antagonist of Beta-Catenin 1 (DACT1) was the only independent prognostic marker of reduced distant bone metastasis-free survival. Preliminary evidence suggests that DACT1, though poorly characterized in the literature, is a Tgf-β-induced upregulator of Wnt signaling, demonstrated by our lab to be active in metastatic cells adjacent to the bone vasculature. Given the implicated role of DACT1 and Wnt signaling in bone metastasis, we then generated a bone metastasis signature using Wnt target genes highly correlated with DACT1 ($R^2 > .25$) both in clinical data sets and bone metastatic cell lines. Interestingly, high expression of the DACT1-derived gene signature had no predictive value for patients with lung, breast or liver metastasis, but was associated with a nearly two-fold higher risk of poor distant bone metastasis-free survival. Together, this evidence suggests that Wnt signaling and DACT1 mediate a bone metastasis-specific program. Ongoing investigations attempt to validate the gene signature and characterize DACT1-dependent activation of Wnt signaling in bone metastasis through in vitro assays and in vivo xenograft experiments.

Exploring mechanisms of spinal curvature in zebrafish models of Idiopathic Scoliosis

Emily Pauls
Dan Grimes
Advisor: Rebecca Burdine

Idiopathic Scoliosis (IS), a disease that causes chronic pain, is characterized by 3D spinal curvatures with severe curves requiring surgery. IS onsets in adolescence and curves are more prevalent in girls than boys. Zebrafish (Danio rerio) have recently emerged as excellent models of IS. Our lab found that defects in motile cilia – an organelle projecting from the cell surface into extracellular space that beats back and forth to generate fluid flow – and cerebrospinal fluid (CSF)-flow cause spinal curves. However, we do not know which ciliated cells are involved nor do we know how CSF flow is sensed in the spine. My work tests the hypothesis that CSF-contacting neurons sense flow using ciliary-localized Polycystin proteins (Pkd2l1 and Pkd1l2a). To test this, I am assessing spinal curve development in mutants in these genes. I have found mild spinal curves in pkd2l1 mutants. By contrast, lethality in pkd1l2a homozygous mutants has thus far prevented robust analysis of IS-like phenotypes. My second goal is to use cell-specific ablation techniques to determine which cell types in the zebrafish brain and spine are required for spinal linearity. To date, I have broadly ablated radial glia and neurons and am observing expected embryonic defects including twitching, early spinal curvature, paralysis, and pericardial edema. Ablations at later time points will assess the requirement of these cell types in spinal linearity. By conclusion, I hope to improve our knowledge of the role of cilia in scoliosis, knowledge which can be applied to refine treatments for those afflicted with IS.
Methylation of lysine-4 of histone H3 (H3K4) plays a crucial role in the regulation of chromatin structure and gene transcription. KDM5B is an enzyme responsible for demethylating this residue, and its dysregulation has been implicated in the proliferation and invasive migration of cancer cells. This enzyme contains a PHD1 finger domain, which has previously been shown to associate with the unmodified N-terminal tail of histone H3. An unpublished finding from our lab suggests that a peptide sequence in KDM5B may mimic this histone tail. Thus, PHD1 may interact with this histone-tail mimic peptide to induce a conformation in KDM5B that intrasterically inhibits the enzyme. However, it is not known whether this interaction takes place and what effect it has on H3K4 demethylation by KDM5B. This current study aims to investigate whether this interaction takes place using $^{1}$H-$^{15}$N HSQC NMR titration. We also examine whether this interaction modulates H3K4 demethylation by KDM5B using immunofluorescent staining of cells transfected with KDM5B mutants aimed to abrogate the interaction. Successful completion of this study will provide insight into the regulation of KDM5B, potentially contributing to therapeutic strategies for the treatment of cancers and other gene regulatory disorders.

The Role of Dopaminergic Terminal Activity in NAc and DMS during Aversive Fear Extinction

Katherine Pizano
Lili X. Cai
Advisor: Ilana Witten

Dopamine (DA) neurons have long been implicated in encoding prediction errors in reward-based learning. However, the role of striatal DA neurons in aversive learning remains largely unknown. We hypothesize that DA terminal activity within the striatum, specifically the NAc and DMS, helps encode a teaching signal which enables extinction learning. To test this hypothesis, we use an aversive fear conditioning behavioral paradigm, along with calcium imaging, to record from striatal DA terminals during extinction. This allows us to analyze how these subpopulations of neurons correlate with conditioned response of freezing to aversive associations. Our preliminary findings suggest DA activity in the NAc and DMS during CS (tone) offset is, in fact, correlated with the aversive action (freezing) within the current trial. Moreover, a decrease in DMS terminal activity at the CS onset also correlates with freezing during extinction trials. Together, both these signals may help drive the conditioned aversive response by preventing extinction learning from occurring. Our next steps will include using optogenetics to manipulate and record from these DA terminals. This will provide causational evidence as to whether dopamine is involved in reinforcing aversive actions or updating the CS-US association. Understanding dopamine’s role during extinction of aversive stimuli can help better our understanding of psychiatric disorders like PTSD, which show strong evidence for the involvement of the DA pathway.
Investigating the Role of Multiple Importin-Binding Sites in the Phase Transition of TPX2, a Critical Spindle Assembly Factor
Aparna Raghu
Raymundo Alfaro-Aco, Matthew King
Advisor: Sabine Petry

The rapid and accurate assembly of microtubules to form the mitotic spindle is necessary to ensure proper chromosome segregation during cell division. This process relies on the coordination of many spindle assembly factors (SAFs). One important SAF in *Xenopus laevis* is TPX2, a protein that promotes microtubule nucleation throughout the spindle. TPX2, in association with the importin α/β complex, can potentially undergo phase transition. The resulting phase droplets may prevent diffusion and delay TPX2 activation until molecules are released through the interaction of importins with RanGTP. To understand the role of importin-binding sequences in TPX2 phase transition, this study will first identify the importin-binding sites of TPX2. Using GST pull-down assays, I will map the nuclear localization sequences (NLSs) of TPX2 by characterizing the interactions between sequential truncations of TPX2 and importins. In addition to the two previously-identified NLSs of TPX2, I predict that other sequences, particularly those identified as putative NLSs *in silico*, will interact with importins. I will then determine whether the presence of multiple NLSs enhances TPX2’s ability to undergo phase transition by mutating basic residues within identified NLSs to alanines, rendering them nonfunctional. I hypothesize that the presence of multiple importin-binding regions helps promote TPX2 phase transition by providing multivalency. This study will provide insight into how the presence of multiple importin-binding domains contributes to phase transition of TPX2. This research will also help inform future studies exploring how phase transition regulates mitotic spindle assembly by concentrating and coordinating activation of TPX2 and other SAFs.

Identification of Reproductive Span Regulating Genes in *C. Elegans* Using Mos1 Insertional Mutagenesis
Rachel Reed
India Rogers-Shepp, Rachel Kaletsky, William Keys
Advisor: Coleen T. Murphy

Reproductive decline in females is one of the earliest signs of aging in the human population. With more women having children later in life, understanding the genetic and molecular causes of reproductive decline is becoming increasingly important. Using the model organism *C. elegans*, a screen can be run using Mos1 insertional mutagenesis to find mutations in the genome that result in extended reproductive span. Mos-1 mediated insertional mutagenesis takes advantage of the capabilities of the Mos-1 transposon of *Drosophila mauritiana*. Using Mos1- mediated mutagenesis, we can mutate the animals, screen them for extended reproduction, then identify and characterize the lesions created by the Mos1 sequence. This protocol allows easy identification of the insertioned mutations in worms that were screened. Although the Mos-1 transposon mutagenesis method has a lower efficiency than classical chemical mutagens, it is advantageous because it provides an opportunity for high- throughput sequencing and mapping, since all mutations would be tagged with the Mos1 gene. The work completed this summer has involved with testing out the protocol and measuring the transposition efficiency of the Mos1 sequence. With the final results of this screen, several key genes will be characterized further to determine their function in extending reproductive span.
How stress can phase out nucleocytoplasmic shuttling

Anastasia Repouliou
David W. Sanders
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To face the growing burden of neurodegenerative diseases, we must develop pathological models. Increasing evidence suggests that the hallmark protein aggregates found in neurodegenerative disease patients result from aberrant liquid-to-solid phase transition of proteins that physiologically phase-separate into liquid-like droplets. During stress, proteins and mRNA are recruited to stress granules (SGs), a class of phase-separating organelles. Nucleocytoplasmic shuttling through the nuclear pore complex (NPC), which is often disturbed in stress, is mediated by nuclear transport receptors (NTRs) that avidly bind to the intrinsically disordered domains of FG-Nups, the proteins guarding the NPC channel. FG-Nups have also been shown to phase-separate into gel-like assemblies, which NTRs traverse like liquids, suggesting NTRs hold a disaggregase in addition to chaperone potential. This study aims to probe the poorly understood interplay between stress response and nuclear transport. We propose that, during stress, SGs sequester NTRs, which act to enable the entry of larger molecules into SGs, while simultaneously disrupting nucleocytoplasmic shuttling. We will therefore test NTR localization to canonical and artificial SGs and to FG-Nups nucleosolic inclusions, and mark changes in the complex body dynamics in NTR overexpression and knockout. Moreover, we will quantify the nuclear to cytosolic ratio of protein and mRNA reporters in the artificial SG-model. The expected results will yield valuable insights into the synergistic transport and disaggregase potential of NTRs, and SG and NPC mechanisms and physiology.

Investigating the Role of DNA Methylation in Stress-Attributed Telomere Attrition

Elizabeth Reznik
Lisa Schneper
Advisor: Daniel Notterman

Epigenetics is the study of covalent modifications to DNA that lead to structural changes within chromatin structure and alter transcription. DNA methylation is the addition of a methyl group to a cytosine that precedes a guanosine, and hypermethylation of CpG islands at promoter regions is associated with inhibition of gene expression. Such genes can include those that regulate the lengths of telomeres, regions of repetitive non-coding sequences at the ends of chromosomes that are implicated in stress-attributed health responses. Few studies have examined the relationship between DNA methylation and telomere length and the molecular mechanism involved. To understand this relationship, data from the Fragile Families and Child Wellbeing Study, a longitudinal analysis of a cohort of approximately 5000 children from 20 cities, will be used to identify candidate promoter regions that are differentially methylated under stressful situations and correlated with telomere attrition. The identified promoter regions will be cloned into pCpGL, a novel luciferase reporter vector that completely lacks CpG dinucleotides on its backbone, thus limiting the effects of DNA methylation on the promoter of interest. By establishing a Dual-Luciferase Reporter Gene Assay, we hope to test the effects of DNA methylation of a promoter region implicated in telomere length regulation on reporter gene expression. This work can provide evidence on a molecular level that chronic stress, particularly at a young age, may predispose an individual to disease via epigenetic modifications.
Creation of a single worm *Mos1* transposon screen for *Caenorhabditis elegans*

India Rogers-Shepp  
Rachel Kaletsky  
Advisor: Coleen T. Murphy

*Caenorhabditis elegans* are a useful model organism for studying both reproductive and somatic aging. Similar to humans, this hermaphroditic worm ovulates for one-third to one-half of their lifespan. Additionally, oocyte development is highly conserved between the two species. Likewise with respect to somatic aging, the Insulin/IGF-1 pathway, TOR signaling, AMP kinases, and sirtuins, have all been shown to be conserved in both species. Discovering the genes responsible for regulating aging is often random. Our lab has proposed to expedite the search for these genes by creating a screen for genes either responsible for longevity. I propose to use a *Mos1* transposon mediated insertional mutagenesis to create a random pool of mutants, followed up with a screen for longevity mutants. I then plan to test individual worms with a *Mos1* screen for single worms to detect the location of the mutation: by testing individual worms I will be able to isolate the strains and study their progeny as well. After many experiments we successfully isolated the *Mos1* transposon element from single worms with a known *Mos1* insert, showing that a single worm contains enough DNA for a genetic screen. Next, I plan to see if aged worms contain enough genetic material for the *Mos1* test. Once I have determined the appropriate parameters for this screen, and I move forward to use it with unknown mutants, this screen will both reduce the amount of time needed to find longevity mutants and significantly increase the known number of longevity genes.

Niemann-Pick Disease (NPC) family proteins in Embryonic Germ Cell Migration

Peyton Smith  
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Advisor: Paul D. Schedl

Proper migration of Primordial Germ Cells (PGCs) in *Drosophila melanogaster* embryos requires attractive and repulsive cues that guide the germ cells to the somatic gonadal precursor cells (SGPs). During embryogenesis, the PGCs migrate through different cell layers in order to associate with the SGPs and form a primitive gonad. Recent studies of Drosophila germ cell migration have shown that the signaling ligand Hedgehog (Hh), the biosynthetic enzyme HMGCoA reductase (Hmgcr), and the ABC transporter Multi-Drug-Resistant-49 (Mdr49) all have roles in the germ cell migration pathway. Because the *mdr49* homologs in mammals (Mdr1/P-cg) provide a cell autonomous function of transporting cholesterol, proteins that are involved in the transport of sequestration of cholesterol, such as niemann-pick disease type C 1 (Npc1) family proteins npc1a and npc1b, are of interest due to the implication of cholesterol's role in PGC migration. Preliminary findings have shown that *npc1a* mutants display germ cell migration defects and that feeding high levels of cholesterol can mitigate migration defects. The proposed experiments aim to confirm and extend the role of Npc1 family proteins in both PGC migration and Hh signal transduction pathway. We will test the model that npc1a and npc1b are involved during transport of ‘cholesterol modified’ Hh ligand in different developmental contexts.
Determination of PqsE active sites in *Pseudomonas aeruginosa*

Camille Sullivan  
Sampriti Mukherjee, Justin Silpe  
Advisor: Bonnie Bassler

Quorum sensing (QS) is a cell density based chemical communication system used by bacteria to control group behaviors. Accumulation of chemicals (autoinducers) secreted by the bacteria coordinate expression of virulence and biofilm genes. This synchronizes bacterial responses to population changes and environmental cues. Bassler laboratory specializes in studying QS pathways and previous work has been done using *Pseudomonas aeruginosa (Pae)* as a model organism. *Pae* utilizes three interconnected QS system pathways to regulate its virulence; one pathway is the PQS pathway, where the autoinducer PQS is synthesized by the enzymes encoded by the *pqsABCDE* operon. The protein PqsE has been discovered to be dispensable for the biosynthesis of the PQS autoinducer. PqsE however is essential for *Pae* QS as a *pqsE* null mutant fails to produce QS regulated public goods such as rhamnolipids and phenazines. How PqsE regulates QS is unknown. Through this project I will determine the residues of PqsE protein that are required for QS. I am performing random mutagenesis of the *pqsE* sequence and observing ensuing levels of the downstream pyocyanin and rhamnolipid production outputs. Mutant strains have been obtained in residues near the N-terminus of the protein and Western blot analyses will be done to determine if the mutants obtained produce stable proteins. A better understanding of the functions and roles of PqsE in QS will introduce new knowledge about the mechanisms of QS which could be used in the future when creating new antibiotics.

An Investigation of Distal Enhancer-Enhancer Communication for the *brinker* Locus in Drosophila  

Mark-Avery Tamakloe  
João Raimundo  
Advisor: Michael Levine

In metazoans, the transcription of many developmental patterning genes is highly regulated, both spatially and temporally, to ensure proper gene expression in the developing embryo. In *Drosophila melanogaster*, these genes often have multiple enhancers that are believed to work in pairs with overlapping activity. Preliminary evidence in the Levine Lab suggests that these paired enhancers do not necessarily work in the widely accepted primary and “shadow” enhancer model, but interact with each other additively. However, most of the studies on these genes have been done *in vitro* and not endogenously, and thus the exact mechanism for how these enhancers communicate is not well understood. To further investigate the potential additive property of enhancers, we performed knockouts of both the early acting and late acting enhancers for the *brk* locus. We found that knocking out either enhancer seems to lead to lethality for half the male population as evidenced by the 2:1 ratio of females to males in both knockout lines. We will temporally monitor the interaction between these two distally located enhancers by tracking the early expression of the *brk* gene and measuring the corresponding levels of nascent *brk* transcripts utilizing the novel live imaging technique of the MS2 reporter system. Understanding the fundamental mechanisms underlying enhancer communication in the regulation of gene expression can provide essential insight in revealing the origin of human diseases that are triggered by abnormalities in the complex multiple enhancer regions.
Understanding outer membrane protein assembly in *E. coli* through suppression of ß-signal mutant LamBG439D

Kimberly Tang  
Betsy Hart  
Advisor: Thomas Silhavy

The outer membrane (OM) of Gram-negative bacteria acts as a first line of defense against toxic molecules in the external environment, including antibiotics. Outer membrane proteins (OMPs) are a major component of the OM that regulates this defense. However, the assembly of the OM and the pathways that regulate its maintenance and formation are poorly understood. In *E. coli*, the Bam complex folds and inserts OMPs into the OM. Previous work has shown that the Bam complex recognizes unfolded OMP substrates by a C-terminal ß-signal sequence. Preliminary work in the Silhavy lab has identified an assembly defective ß-signal mutant in the maltoporin LamB, *lamBG439D*, that is suppressed by the deletion of the periplasmic protease *degP* at 30ºC. Intriguingly, *degP* deletion is lethal in this background at 37ºC, suggesting that unfolded substrate accumulates in the periplasm and creates a toxic backup of unfolded OMPs. This temperature sensitivity has allowed us to select for suppressors at the non-permissive temperature of 37ºC that are able to overcome the toxic accumulation of unfolded OMPs. Furthermore, we have utilized MacConkey indicator media to detect LamB assembly in these suppressors using dextrin uptake as a readout. Using this approach, we have isolated twelve suppressors of *lamBG439D* that will provide the opportunity to understand the interactions that take place on the OMP assembly pathway.

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Investigating the Relationship of Fibronectin and LOX Family Proteins during Matrix Assembly

Sarah J. Tian  
Katherine E. Hill  
Advisor: Jean E. Schwarzbauer

Fibrosis is characterized by the excess deposition of extracellular matrix (ECM) components in an organ or tissue and increased cross-linking. Fibronectin (FN) is a key glycoprotein in ECM and regulates cell function in conjunction with other ECM proteins such as collagen. Furthermore, collagen matrix assembly is dependent on the presence of fibronectin. The lysyl oxidase (LOX) enzyme is responsible for collagen cross-linking during matrix formation. Two isoforms, LOXL1 and LOXL2, are also known to have cross-linking functions. Presently, the effect of the fibronectin matrix on collagen matrix assembly is not well understood. This study aims to understand the relationship between FN and LOX family proteins in an effort to clearly elucidate a role for the enzyme in matrix assembly. HT1080 fibrosarcoma cells were treated with dexamethasone to stimulate FN matrix assembly. HT1080s were shown to express LOX and LOXL2, but not LOXL1. mRNA expression of FN and LOX family proteins is measured by qPCR. Stimulation with dexamethasone caused a change in expression levels. A time course was developed to show how expression levels differed after 24, 48 and 72 hours of treatment with Protein expression will be quantified by Western blot at a later time. This study will provide insight into the interaction between FN and LOX family proteins which prove useful as a target in the therapeutic treatment of fibrotic diseases and cancer progression.
Establishing a Mechanism for Stress-Induced Telomere Shortening in Human Foreskin Fibroblasts

Ava Torjani
Lisa Schneper, Iulia Kotenko
Advisor: Daniel Notterman

Stress is associated with harmful outcomes including depression, cancer and cardiovascular disease. Previous studies have shown that the stress hormone, hydrocortisone, accelerates telomere attrition in human foreskin fibroblasts (HFF) and reduces telomerase activity in human T-lymphocytes. However, the link between telomerase activity and telomere length is unclear, and the mechanism behind the effects of stress on telomerase activity is unknown. This study aims to determine the effect of hydrocortisone treatment on telomere length and the mechanism of how hydrocortisone alters telomerase activity in HFF cells. Previously, the laboratory showed that exposure of HFF cells to hydrocortisone decreases telomerase activity and alters expression of telomere maintenance genes. The current study will confirm these findings by examining the effect of hydrocortisone on HFF telomerase activity using Telomere Repeat Amplification Protocol (TRAP), and subsequently on telomere length using qPCR. Additionally, altered expression of potential hydrocortisone targets, including non-coding RNA (HSAT-II), and the telomerase components, the RNA template (TERC) and reverse transcriptase (TERT), will be examined by qPCR. We confirmed a decrease in telomerase activity in response to hydrocortisone, warranting further research to confirm significance. We also expect that hydrocortisone treatment will be associated with shorter telomeres and differential expression of selected targets. Future experiments will examine the role these targets play in altering telomerase activity in response to hydrocortisone using RNAi. These results will provide a foundation to elucidating the mechanism behind stress-induced telomere shortening.

roX1 and roX2 Chromatin Entry Sites: Dosage Compensation and Boundary Elements

Aidan Waugh
Amina Kurbidaeva
Advisor: Paul Schedl

Different organisms use different mechanisms to equalize expression of X chromosome genes. In Drosophila melanogaster, transcription of X-linked genes in male flies (X/Y) is upregulated to match the level of expression in females (2X). The Male specific lethal complex (MSL) is responsible for dosage compensation in males. The MSL complex solely associates with active genes on the X-chromosome. Key to targeting the MSL complex to the X- chromosome are specific loci called Chromatin Entry Sites (CES). It’s thought that there are more than 100 CES along the X chromosome and that MSL complexes are initially recruited to these loci and spread to nearby genes. The LBC protein complex binds to several different CES, including the canonical roX1 and roX2 CES. Since LBC is also a component of a Bithorax complex boundary (insulator) element called Fab-7, this discovery raised the possibility that roX1 and roX2 could also act as boundary elements. Boundary elements define the 3D chromosomal topology by pairing to each other and this can be demonstrated using “transvection” assays. In collaboration with the Jaynes lab (Thomas Jefferson University) we generated a set of attP transgenes containing roX1 or roX2 CES flanked either by enhancers or lacZ and GFP reporters. I’ve generated stage 13 embryos carrying the enhancer/CES transgene on one homolog and the reporter/CES transgene and probed for lacZ expression using a smFISH procedure followed by confocal microscopy. I’m refining my protocols in order to improve the image quality. We hope to show that roX1 and roX2 loci display pairing activity similar to known Drosophila insulators. This would help us learn more about the topology of the X chromosome and MSL spreading mechanism.
Gram-negative bacteria have cell envelopes made up of an inner membrane, a peptidoglycan cell wall, and an outer membrane. The outer membrane is made of lipopolysaccharides, β-barrel proteins, lipoproteins, and phospholipids. The mechanisms of trafficking lipopolysaccharides, β-barrel proteins, and lipoproteins are well studied and understood. However, there is a gap in knowledge regarding the trafficking of phospholipids. *E. coli* inner membrane protein YejM is homologous to the cardiolipin transporter PbgA in *Salmonella*, and is essential even though cells can survive without cardiolipin. Previous studies have associated mutant yejM with defects in the outer membrane. The current study aims to investigate the possible role of YejM in phospholipid trafficking. A truncated version of yejM is temperature sensitive at 42°C. Spontaneous suppressors of the mutant yejM temperature sensitivity phenotype are currently being isolated. These mutations in associated genes will be identified and characterized to better understand the function of yejM. I am also recombineering a new strain with a yejM truncation carrying a chloramphenicol marker at the truncation site. Once constructed, this strain will be used with the EZ-Tn5 transposome to create a library of transposon insertions. Transposon insertion sequencing will then be used to identify genes that are synthetic lethal with mutant yejM. This study is undertaken to better understand phospholipid trafficking to the outer membrane of Gram-negative bacteria. Phospholipid trafficking is the last largely unknown component in assembly of the outer membrane, and is important for basic biological knowledge of bacterial development and may be useful in targeting infectious bacteria.

Cholera is the leading diarrheal disease worldwide and is caused by the pathogenic bacterium *Vibrio cholerae*. *V. cholerae* has a characteristic curved rod shape that provides an advantage for swimming motility and pathogenesis. The degree of curvature is dynamic, and it can be perturbed by mutation of the curvature determinant crvA or growth in high osmolarity media; however, the effects of other environmental conditions on *V. cholerae* have not been thoroughly investigated. The objective of this research is to discover new environmental conditions that change the dynamics of *V. cholerae* curvature and to understand their underlying mechanisms, with an ultimate goal of uncovering how these conditions change *V. cholerae* life cycle. By screening *V. cholerae* curvature in a variety of growth conditions, I have identified new carbon sources that alter curvature. Preliminary data show that growth rate may contribute to curvature changes. These findings also strengthen the negative correlation between osmolarity and curvature. Since motility is important for *V. cholerae* virulence, future motility assays will be performed on *V. cholerae* grown under the curvature-altering conditions. This research will better inform the scientific community on the curvature dynamics of *V. cholerae* and its relationship to pathogenic behavior. These results can potentially offer insight on how to target virulence in bacteria based on cell shape without selecting for resistant bacteria with antibiotics.