MOL CLASS OF 2022 SENIOR THESIS ABSTRACTS

Identification and characterization of positive regulators governing hepatitis C virus host tropism

Maricar Almeda
Advisor: Alexander Ploss

Hepatitis C virus (HCV) continues to affect millions of lives worldwide. Although the advent of highly effective direct-acting antivirals (DAAs) has revolutionized clinical management of chronic hepatitis C as the great majority of affective individuals can be cured of their infection, DAAs are very expensive and HCV transmission remains high in most countries, including the United States. Therefore, the development of a vaccine is essential to contain HCV transmission more efficiently. However, systematic testing of vaccine candidates is significantly hindered because HCV can only infect humans and chimpanzees. Understanding HCV's limited host tropism is critical towards the development of an animal model for HCV infection. This study hypothesizes that human-specific host factors exist which are not expressed in murine cells or whose murine orthologues are not functional, that can boost HCV permissiveness in murine cells. Prior studies have implicated Tripartite Motif Containing 26 (TRIM26), and cyclophilin A (CypA) to modulate HCV replication efficiency across different species. However, our results do not confirm that their expression augments HCV infection in murine cells. By utilizing the human ORFeome library, a gain of function screen was conducted to identify additional human factors that augment HCV infection in murine cells. In parallel, transgenic mice were generated to express human factors and infected with HCV to assess the effect of these human factors on HCV infection in vivo. Identifying and characterizing positive regulators that govern HCV host tropism will lead to the construction of an HCV-susceptible mouse that can be used for further research which will pave the way for the development of a vaccine for HCV.

Gender-Affirming Hormone Therapy for Transgender Youth: Telomere Homeostasis, Psychological Wellbeing, and Barriers to Research

Joshua Babu
Advisor: Daniel Notterman

Transgender youth face uniquely high rates of psychological distress. Such stress – particularly when prompted by childhood adversity – has been well-documented as concomitant with accelerated telomere shortening and depressed telomerase activity, which increase genomic instability and susceptibility to numerous health concerns. The relationship between stress and telomere length has not yet been studied in trans youth, nor has there been any research on whether telomere homeostasis in trans youth is impacted by gender-affirming hormone therapy (GAHT), which is a notably understudied field of medicine. Thus, we set out to (1) use PCR-based analyses of telomerase activity and hTERT expression to determine the effects of sex hormones on telomere homeostasis in vitro; (2) establish an infrastructure for a larger clinical study of trans youth undergoing GAHT that assesses longitudinal changes in physiological and behavioral parameters, including telomere homeostasis and psychological wellbeing; and (3) examine the procedural and systemic barriers to our clinical study using a continuing quality improvement (CQI) approach. Validated by repeat measures and the use of appropriate controls, our in vitro analysis revealed that telomerase is post-translationally upregulated by β-estradiol and testosterone, thus establishing a baseline biological relationship between telomere homeostasis and steroid hormones in normal human somatic cells. This will complicate any results derived from our future in vivo analyses of telomere homeostasis in trans youth receiving β-estradiol or testosterone treatment. Establishing a foundation for our larger clinical study, we successfully formed an interdisciplinary research team, recruited a cohort of patients undergoing GAHT, validated the

functionality of the surveys used for their psychological assessment, and initiated the formation of a biological repository for use in in vivo analyses of their telomere homeostasis. Finally, our CQI investigation revealed 7 major areas of concern in our patient recruitment strategies. This allowed for the implementation of reforms that will not only improve our own methodologies but also provide insight into best practices for future clinical research on GAHT for trans youth. Amidst a notably volatile sociopolitical climate around GAHT, this thesis serves as a roadmap for establishing reliable research infrastructure for investigating the longitudinal impacts of gender-affirming medical care on the wellbeing of trans youth, both at the macroscopic and molecular levels.

Analysis of the Impact of Site-specific Demethylation of N6-methyladenosine on Interferon β

Daniel Beard
Advisor: Alexander Ploss

Epitranscriptomics is a growing field of study that refers to the factors outside of genes that affect expression via direct regulation of RNA molecules, by chemical modification. The most abundant and well-studied RNA modification is the methylation of the N6 position on adenosine, or N6-methyladenosine (m6A). The placement of this modification is mediated by the methyltransferase complex, METTL3 and METTL14. Our lab has amassed data demonstrated that the targeted disruption of METTL14 in mouse hepatocytes in vivo causes liver damage, including fibrosis and cirrhosis. It is presently unknown which specific transcript(s) – being subject to epitranscriptomic regulation – are responsible for the observed phenotype. To enable demethylation of specific transcripts, we constructed a CRISPR dCas13-FTO construct that can be directed to specific sites using a guide-RNA. As a proof-of-concept, we target interferon-β (IFNB), which has been well studied in its relationship with METTL14 depletion in the liver, to demethylate three m6A modified sites on its RNA transcript. Ultimately, vector-mediated delivery of CRISPR dCas13-FTO can be used to specifically remove m6A modifications from other RNA transcripts in the liver that are up- or downregulated in response to METTL14 depletion.

Investigating a Link Between CrvAB and Bacterial Pathogenesis Across Vibrio Species

Mina Beshy
Advisor: Fred Hughson

Membrane fusion is required for transport of material between organelles and to the extracellular space in eukaryotic cells. SNARE proteins, anchored across membranes, facilitate fusion by zippering into four helix bundles. Although SNAREs are necessary and sufficient for membrane fusion, they function slowly without the aid of other proteins. In vivo, additional protein machineries like Rab GTPases and tethering proteins regulate membrane proximity, while Sec1/Munc18 (SM) proteins catalyze trans-SNARE complex assembly. By functioning both as a tethering factor and SM protein, the HOPS complex regulates all membrane fusion in the endolysosomal pathway. To accomplish these functions, HOPS contains two Rab-binding subunits, which allow it to tether two membranes in space. HOPS' SM protein subunit, Vps33, then catalyzes SNARE complex assembly by templating SNAREs and fusing the two membranes. The precise mechanism by which HOPS is activated to perform its functions, though, is unknown. Structural biology has been used to gain insight about HOPS' mechanism of action. Work done by a member of our lab yielded a 4.7 Å resolution cryo-EM structure of HOPS' SM protein subunit Vps33 in complex with Vps18 and Vps16, two other HOPS subunits. The structure showed surprising evidence of Vps18 binding within an important SNARE binding active site of Vps33, inspiring the hypothesis that Vps18 is an allosteric regulator of Vps33. To characterize the Vps33:Vps18 binding interaction, we sought to use biochemical assays and x-ray crystallography to validate the existing cryo-EM structure and the interactions predicted by it. The data presented in this study suggests that while the cryo-EM structure generally maps Vps18 properly within the

Vps33 active site, the specific residue interactions modeled in the structure need further biochemical and structural validation.

Branching Morphogenesis and the impact of Canonical WNT on Mechanical and Dynamic properties of the Lung Mesenchyme

Adam Boukind
Advisor: Celeste Nelson

Congenital lung disorders are a leading cause of infant mortality and cause high morbidity in adults. A better understanding of lung morphogenesis is key to improving current treatment options and will pave the way for future tissue engineering applications. Branching morphogenesis of the mammalian lung is tightly controlled by a feedback loop of coupled interactions between the airway epithelium and surrounding mesenchymal and mesothelial tissues. Patterning of the mesenchyme is of particular interest given its critical role in sculpting the epithelium. Fibronectin deposition and airway smooth-muscle differentiation at mesenchymal cleft sites act as a physical barrier to induce terminal bifurcation of growing branches. While research suggests that canonical WNT signaling plays an important role during lung development, its precise function in the mesenchyme during terminal bifurcation of epithelial branches is still under investigation. This study seeks to investigate how canonical WNT influences branching through changes in the mechanical and dynamic properties of the embryonic pulmonary mesenchyme. By modulating canonical WNT using pharmacological inhibitors and activators, we show that canonical WNT signaling plays an important role in the differentiation and localization of airway smooth muscle around the epithelium. This project also hints at the redundancy of mechanisms by which mesenchymal WNT signaling regulates branching through FN and Actin remodeling.

THE MOLECULAR BIOLOGY OF SARS-COV-2 AND ITS EVOLUTION WITHIN THE PRINCETON COMMUNITY

Jesse Brewer
Advisor: Daniel Notterman

SARS-CoV-2, a novel and highly pathogenic coronavirus, has caused unprecedented global disruption following its introduction into the human population. From the first individual's falling ill with COVID-19 (the disease resulting from SARS-CoV-2 infection) in December 2019, the pandemic has claimed millions of lives and caused nations around the world to implement drastic public health measures. College campuses across the United States have executed numerous strategies aimed towards reducing the spread of COVID-19 through mask-wearing, social distancing, asymptomatic testing, and contact tracing. Beginning in the 2021 spring semester, Princeton University invited all students to campus and initiated an asymptomatic testing protocol using PCR-based detection of human saliva samples. RNA extracted from PCR-positive human saliva samples was sequenced for surveillance purposes. Using this sequence data, I monitor the evolution of SARS-CoV-2 and emergence of variants over time at Princeton University. Further, in the SARS-CoV-2 Variant Detection project section of this thesis, I optimize a CRISPR-based detection assay for a cluster of eight B.1.1.7 lineage variants possessing a 48-nucleotide deletion in the 3'- UTR of the SARS-CoV-2 genome, eight ancestral B.1.1.7 lineage variants, and validate these assays on Princeton University de-identified extracted SARS-CoV-2 RNA from human saliva samples. Thus, in this senior thesis, I: 1) monitor and characterize the emergence and spread of SARS-CoV-2 variants at Princeton University, 2) provide evidence of the SARS-CoV-2 transmission chain at

Princeton University between January 25, 2021 and March 1, 2022, and 3) report my work to develop, optimize, and validate a CRISPR-based detection assay for two B.1.1.7 lineage variants.

Exploring the Structural and Biochemical Characteristics of the SM Protein Sly1 in Complex with Associated Qa-SNARES Ufe1 and Sed5

Christian Brown
Advisor: Fred Hughson

Vesicular trafficking is controlled by the precise interaction of Rab proteins (G-proteins) and the cell cytoskeleton to deliver important material into and out of the cell and cell organelles. The focus of this paper is the very last step in the process; vesicle and target membrane fusion. This process is carried out by soluble N-ethylmaleimide sensitive factor attachment protein receptors (SNAREs) which are the necessary and sufficient machinery for inducing membrane fusion. Although SNAREs are sufficient for this process, it occurs at a slow rate. Cells utilize proteins called Sec1/Munc18-like proteins that expedite this process by speeding up SNARE complex formation. Relatively few structures are available that inform us on how SM proteins bind with and transform SNAREs. For the SM protein Sly1, the only published crystal structure is a complex containing a small N-terminal peptide derived from the Qa-SNARE Sed5. This study sought to produce a structure of the Sly1-Sed5 complex containing a more complete Sed5, as well as a structure of Sly1 in complex with a similar SNARE protein named Ufe1. These structures will provide insight into the mechanism(s) whereby SM proteins regulate SNARE assembly.

cfap298/Kurly Functions to Orient Centrosomes in the Early Zebrafish Embryo

Tyler Bruno
Advisor: Rebecca Burdine

Polarized cell processes, such as those that allow for the coordinated movements of the cells in the embryo, are important for proper development. cfap298/c21orf59/Kurly is a protein previously identified for its role in maintaining polarization and proper function of motile cilia. However, kurly is expressed in cells that do not have motile cilia; we hypothesize that Kurly may affect other polarity events in these cells. In this study, we sought to determine how Kurly functions in polarized developmental events in contexts that are devoid of motile cilia. In cleavage stage kurly mutant embryos, we observe irregular cellular divisions and improper centrosome positioning and organization in kurly mutants, indicating that kurly affects polarization of centrosomes at these stages. These early defects did not affect embryo survivability: convergence and extension, a polarized gastrulation movement, was unaffected, and mutant embryos were able to sustain development to adulthood. To explore where Kurly protein functions at a subcellular level, various tools were designed and assessed in attempts to visualize kurly. Our results suggest that Kurly may function as a scaffold that recruits centrosomal organizing proteins to the centrosome and maintains other polarized developmental events.

Investigating the Effects of FGF Signaling on Prdm1 Expression in *Ciona intestinalis*

Emily Chavarria
Advisor: Michael Levine

Understanding how gene regulation is controlled is one of the most fundamental factors to understand how embryos development and disruptions to gene regulation can result in diseases. Prdm1 transcription factors are known to be important in Ciona development as well as in human immunologic responses. However, the gene

regulatory interactions remain unclear in both models. In this study I used reporter assays and HCR in situ hybridization to understand Prdm1 expression within early Ciona. I found that for 2 of the Ciona Prdm1 genes, Prdm1-r.a/b, expression is upregulated by FGF signaling. ETS and GATA motifs, highly conserved genomic regulators, are clustered in the enhancer regions of Prdm1-r.a/b and were sufficient in driving expression and response to FGF, establishing them as important avenues of study for understanding the regulation and functional role of Prdm1 in Ciona and other organisms. Further work would be needed to done to further understand the temporal regulation and differences between Prdm1-r.a and b. Based on analysis of minimal enhancer regions in both Prdm1 paralogs, I propose the pioneer factor Foxa.a as a possible temporal regulator for Prdm1-r.a in Ciona.

Analysis of *Drosophila Virilis* Chromatin Structure and its Formation During Early Embryogenesis

Marcus Curlin
Advisor: Michael Levine

The 3-dimensional organization of the Drosophila virilis genome and the mechanism of its development are poorly understood. A series of technological advancements over the past decade have revolutionized the ability to study chromatin structure in the cell. In this thesis, I analyze D. virilis Micro-C and ATAC-seq data during embryogenesis to better understand the patterns of chromatin architecture and the genetic elements involved in its formation. By comparing ATAC-seq chromatin accessibility data between nc14 and later mixed-stage D. virilis embryos, I identified a number of potentially regulated genes during embryogenesis. I propose that these putative cis-regulatory regions can be useful targets for investigation on changes in chromatin structure during early embryogenesis. Additionally, I identified a pattern of heightened chromatin accessibility at topologically associating domain (TAD) borders, especially during nc14, implicating the possibility of genetic elements involved in TAD formation. Separately, I helped optimize the RD-SPRITE protocol for Drosophila virilis embryos, setting up future research to understand the role of DNA-RNA interactions in establishing 3D genomic structure during early embryogenesis.

Analyzing the Prevalence of Newly Discovered Bacterial Defense Genes and Approaches to their Quantification

Mary Davis
Advisors: Mohamed Abou Donia and Ned Wingreen

Bacteria possess a variety of defense systems to protect themselves from bacteriophage infection. These systems have been exploited to create widely used tools in both experimental and therapeutic contexts. Defense system genes tend to be clustered on the genome within 'defensive islands.' By searching for genes overrepresented on those islands, an enormous variety of defense systems were recently discovered. The Prokaryotic Antiviral Defense System (PADS) Arsenal is the first database to incorporate these newly discovered systems into a comprehensive list of all bacterial defense genes. This thesis analyzes the defense genes identified in the PADS Arsenal and the efficacy of the PSI-BLAST quantification tool for future defense gene identification. Analysis of the PADS Arsenal in 62,164 bacteria uncovered a high prevalence of defense system genes and a tendency for these genes to co-occur in the same genome, which is partially dependent on genome length. Despite their high prevalence in general, this analysis also identified a small number of bacterial genomes with no known defensive genes. The PSI- BLAST search tool demonstrated low recall and precision in identifying "cyclase," a defense gene from the novel cyclic-oligonucleotide-based anti-phage signaling system. Transitioning to matrix-based search methods and focusing identifications of defense mechanisms on complete systems will likely increase the recall and precision of future defense system quantifications. Our findings provide a comprehensive view of the distribution of anti-phage defense systems in 62,164 bacterial genomes and evaluates currently used methods for their identification and quantification.

Investigating the Applicability of Immune tSH2-ITAM Binding as a Molecular Biosensor for FGFR1 RTK Activation

Kaylan Fomby
Advisor: Jared Toettcher

Receptor tyrosine kinases are important for studying cell signaling pathways. Their binding activity to extracellular signaling proteins at the plasma membrane activates phosphorylation cascades that induce the downstream expression of target genes involved in various cellular processes including cellular proliferation and differentiation. Unfortunately, we lack biosensors that successfully report specific RTK activation patterns in single living cells. Therefore, in this paper we sought to better understand the dynamics of RTK activation through the development of an efficient RTK-specific biosensor capable of reporting real-time activation of these receptors at the plasma membrane. Based on our understanding of orthogonal binding of the ZAP70 protein tSH2 domain to ITAM regions of transmembrane T cell receptors, we inserted a tSH2-ITAM reporter system into single living cells to act as a biomarker for ZAP70 protein binding at the pTyr residues of FGFR1 upon being activated by FGF ligand stimulation. We saw RTK activation efficiently reported as visualized by swift localization of ztSH2 to the FGFR1-ITAM construct localized at the plasma membrane. However, the absence of a negative control with cells lacking the tSH2-ITAM system necessitates further validation of the biosensor. The results implicate a potential generalizable, live-imaging biosensor capable of reporting multi-RTK activation patterns to highlight new understanding of cell signaling dynamics and their connections to cell proliferation and many other important cellular processes.

Investigating the Divergence of Cerebellar Modules using Transsynaptic Viral Tracing and High Throughput Whole Brain Imaging

Samuel Frank
Advisor: Samuel Wang

The cerebellum plays an important role in motor and cognitive functions. In addition, cerebellar abnormalities are strongly associated with developmental disorders such as autism spectrum disorder. To understand the role of the cerebellum in these disorders it is important to first have a strong understanding of how the cerebellum is organized in its typical state. Decades of research has shown that cerebellar computations occur within parallel modules. However, the extent to which these modules are closed off from other modules has not been studied in depth. In this thesis, we applied modern transsynaptic tracers and high throughput whole brain imaging to see if modules form closed circuits. To do this, we performed a novel disynaptic tracing experiment using Herpes Simplex Virus (HSV)-129 ΔTK-TT and analyzed disynaptic projection patterns in previously collected HSV-129 and Pseudorabies virus (PRV)-Bartha datasets. Convergent evidence across these three experimental approaches indicate that disynaptic projections diverge from what would be predicted if modules were completely closed. Together these findings suggest that cerebellar circuits are not entirely closed on a modular level. In summary, this work clarifies the degree of divergence in the cerebellar trisynaptic loop and contributes to imaging, viral, and annotation approaches for anatomical probing of cerebellar circuitry.

Mechanisms of Asymmetric Cell Division in the Early Mammalian Embryo

Zsombor Gal
Advisor: Eszter Posfai

Preimplantation development features the segregation of trophectoderm (TE) and inner cell mass (ICM) progenitors in the mammalian embryo. Differential activation of TE and ICM cell-fate-specific gene expression programs have been linked to the presence or absence of apicobasal polarity, respectively, in blastomeres of the 16-cell-stage embryo and beyond. Polarity differences are first set up at the 8-16 cell transition by asymmetric divisions of polarized 8-cell-stage blastomeres. This symmetry-breaking event was thought to proceed via the asymmetric inheritance of the apical domain (AD), though recent findings suggest the AD may disassemble prior to division. These observations have therefore raised many questions about the mechanistic basis for asymmetric polarity inheritance. Here in this thesis, I test the hypothesis that asymmetric inheritance of the basolateral domain ensures the asymmetric polarity of daughter cells. First, via the live imaging of mouse embryos, I confirmed that the AD is disassembled during the 8-16 cell transition. Then, by developing and validating reagents to visualize the dynamics of basolateral domain proteins, the localization and inheritance patterns of three conserved basolateral factors (SCRIB, LLGL1, and E-cadherin) were assayed. I discovered that SCRIB was retained at the membrane during mitosis, and that its overexpression inhibited establishment of apical polarity. This latter finding points to possible antagonism between regulators of apical and basolateral polarity. Furthermore, I revealed that E-cadherin is retained at basolateral regions during mitosis, and that it undergoes asymmetric inheritance at the 8-16 cell transition. Finally, I showed that AMOT, a key factor that links polarity to cell-fate-specific gene expression through regulation of the Hippo pathway, is symmetrically inherited at the 8-16 cell transition, despite its apically-polarized localization at the 8-cell stage. Together, this work outlines a novel model whereby asymmetric inheritance of basolateral domain components may differentially regulate the establishment of polarity in daughter cells at the 16-cell stage. Additionally, I propose that disassembly of the AD may be required for symmetric AMOT inheritance, which in turn may be necessary for timely activation of cell-fate-specific Hippo signaling. In summary, this work provides novel insights into the first symmetry-breaking event during mammalian development.

RECONSTITUTION OF THE HUMAN R-TYPE Cav2.3 CHANNEL INTO PROTEOLIPOSOMES FOR CRYO-EM STRUCTURAL ANALYSIS

Sarah Gao
Advisor: Nieng Yan

Voltage-gated calcium (Cav) channels are a family of transmembrane proteins that mediate calcium entry into the cell by responding to changes in membrane potential. They are found in many types of excitable cells and regulate key physiological processes through membrane-dependent channel gating. In particular, the human R-type Cav2.3 channel is strongly expressed in neuronal and neuroendocrine cells, elevating its pharmacological importance as a therapeutic target for neurological diseases like epilepsy, pain response, and Parkinson's disease. However, due to the resistance of Cav2.3 channels to most selective inhibitors, little is known about the modulation and inactivation of these channels for rational drug design. Moreover, the molecular mechanisms of Cav channel gating processes are unclear, as detailed structures of most channel states have not yet been elucidated. Recently, high-resolution structures of the human Cav2.3 complex were determined by the Yan lab, and correlated functional studies were performed to gain insight into the inactivation gating mechanism of Cav channels. The goal of the current study was to further image Cav2.3 channels in native-like environments, through the development of a reproducible method for reconstituting human Cav2.3 into proteoliposome systems and preparing high-quality cryo-samples for 3D structural reconstruction. Our method lays a strong

foundation for uncovering the complete working mechanisms of Cav channels through structural elucidation of their various physiological states.

Developing new methods: Adapting the CRISPR-Cas13 genome editing system to study germ granule mRNAs in *Drosophila* germ cell development and beyond

Zoya Gauhar
Advisor: Elizabeth Gavis

Early in development, cells undergo a decision between germline and somatic fates. In Drosophila, the germ cell progenitors, called pole cells, form at the posterior of the embryo and contain the maternally derived germ plasm. Germ plasm contains protein-and-mRNA- rich assemblies called germ granules that promote the germ cell fate. As effective methods for knocking down transcripts locally are lacking, the functions of many germ granule mRNAs in germ cell development remain a mystery. This thesis reports a new methodology, using CRISPR-Cas13, for knocking down transcripts efficiently in a localized manner in Drosophila. I selected two germ granule transcripts, one encoding the regulator of calcineurin, Sarah (Sra) and one encoding the actin nucleation factor, Spire (Spir). I transgenically expressed CRISPR RNAs (crRNAs) targeting these transcripts in flies. I generated lines with different Cas13 transgenes that control Cas13 expression on demand. The system will be used to investigate Sra and Spir function in promoting germ cell development. This thesis also reports a Cas13-based method for live imaging of RNA dynamics. These technologies will be optimized to be relevant for other scientists. To conclude, I shift from the microscopic to the global scale to evaluate the outlook of CRISPR technologies for treating diseases.

Towards a novel single-cell transcriptome-profiling technique: Investigating the capacity of CRISPR RNAs to prime reverse transcription

Christopher Guan
Advisor: Cameron Myhrvold

Single-cell RNA sequencing has revolutionized our understanding of molecular biology and has become a ubiquitous tool in laboratories across the world. However, RNA sequencing library preparation still presents a technological obstacle. To alleviate these challenges, scRNA-seq methods utilize cell-specific transcriptome-profiling methods to allow sequencing readouts of each transcript to retain the molecular memory of the cell from which they are derived. This process enables the pooling of multiple distinct cell samples for parallel library preparation. However, multiplexing methods typically demand that cells are separated into independent indexing environments using time-consuming or resource-intensive methods, thereby presenting a technological challenge. Here we propose a unique barcoding CRISPR-Cas13-based technique that utilizes cellular machinery for transcriptome-profiling, which could dramatically reduce costs and accelerate the library preparation process. This thesis explores the molecular mechanism by which CIRSPR-Cas13 profiles the transcriptome. We show that CRISPR RNAs are sufficient for priming reverse transcription of specific transcripts both in vitro and in situ. We then show the efficiencies of priming reverse transcription by crRNAs across varying reaction conditions. Finally, we demonstrate that CRISPR RNA spacer sequences can be designed to indiscriminately target polyadenylated mRNAs for reverse transcription. In sum, we established the groundwork for continued exploration, optimization, and development of a novel cell-specific transcriptome-profiling mechanism.

Characterization of the function of m6A regulation in breast cancer

Arjun Guthal
Advisor: Yibin Kang

Breast cancer is currently the most diagnosed cancer in women and classified among the most metastatic cancers. Specifically, breast cancer originates within the epithelial cells of the mammary gland, a highly dynamic and stem cell abundant region that develops extensively through puberty and pregnancy. In recent years, researchers have begun focusing on how mammary stem cells (MaSCs) are regulated during mammary gland development and breast cancer progression. One approach involves investigating the role of internal RNA modifications in regulating breast cancer maintenance. N6-Methyladenosine (M6a) is currently the most abundant internal mRNA modification, however despite being highly conserved, researchers are conflicted over its functional significance in disease. The discovery of m6A regulating proteins has been a significant achievement in this area of study, with recent emphasis being placed on the role of m6A "readers" in embryonic development, regulating proteins that interpret and direct m6a methylated transcripts accordingly. Among the m6A readers, YTHDF2 (DF2) has been recently shown to have irreplaceable importance in the regulation of m6A, yet it remains largely uncharacterized in breast cancer. In this study, we examine the role of DF2 within the context of breast cancer, specifically by testing its effects on tumor cell proliferation, stemness, and metastasis in vitro. We find that upon knockdown of DF2 in human and mouse mammary tumor cells, overall tumor cell proliferation, stemness, and invasion all increase, a finding that was supported by preliminary bioinformatics analysis of m6A expression in clinical breast cancer dataset samples. Additionally, DF2 KD cells showed increased expression of transcription factors essential to maintaining the stem cell phenotype, suggesting a role for DF2 to normally downregulate stem promoting transcripts. Although more research is necessary to determine how DF2 may be regulating processes such as stemness or metastasis, our results suggest a possible tumor suppressor-like role within the context of breast cancer.

Methods for Engineering a Light-Switchable CRISPR-Cas13 System

Jack Hariri
Advisor: Jared Toettcher

CRISPR-Cas systems have emerged as a highly promising biotechnological tool in recent years. The Cas13 subfamily of CRISPR/Cas proteins act differently than the usual CRISPR-Cas9 system by cutting RNA, rather than DNA. This offers a promising approach to regulate transcript abundance without permanent genetic modification, with applications in both basic science and therapeutics. Here, in collaboration with the Myhrvold laboratory, we propose and test two methods for engineering a light-switchable Cas13 to control RNA cutting precisely over time and in specific cells in a tissue. The first of these methods is the addition of a stem loop to the Cas13 gRNA that can bind the light-activated PAL protein in light conditions, potentially blocking Cas13-gRNA binding. We discovered that modifications to the gRNA can be made without blocking Cas13 function on its own, however the addition of the PAL protein is not capable of conferring light-switchable properties onto Cas13 using the current system design and with PAL binding protein expressed at the tested levels. The second of these methods is the insertion of a light-switchable LOV2 domain at different sites in the cas13 sequence, following recent successes in engineering light-switchable nanobodies and monobodies, which can be followed by a high-throughput screening to test for light-switchable properties. These results indicate that insertion of the LOV2 domain throughout the CAS13 gene can be performed using transposition followed by a procedure known as Golden Gate Cloning. However, modifications to the procedure must be made so that

successful insertions can be isolated and tested for light-switchable properties. These tests serve as early evidence that engineering a light-switchable CRISPR-Cas13 is possible, laying the groundwork for a procedure that will allow for tightly controlled degradation of any user-defined RNA in space and time.

High Throughput Localization and Characterization of Conserved Photosynthetic Proteins in *Chlamydomonas Reinhardtii*

Henry Harrigan
Advisor: Martin Jonikas

An ongoing food crisis affects humanity, and today millions face hunger worldwide. Demand for foodstuffs is projected to exceed supply within decades, yet climate change and deforestation are set to diminish the land suitable for agricultural use. One solution, already met with success, is increasing crop yield efficiency through bioengineering staple crop strains. Model green algae Chlamydomonas Reinhardtii performs a highly efficient mode of photosynthesis within unique subcellular structures. Crop strains performing photosynthesis similarly efficiently would constitute a technical achievement and resecure the agricultural sector. Chlamydomonas remains the most well understood alga, yet reverse engineering attempts are limited by a poor understanding of its chloroplast. Evolutionarily conserved genes common to land plant model Arabidopsis Thaliana offer an untapped wealth of information that informs models in both organisms. Informed by literature, we selected 289 Chlamydomonas genes whose Arabidopsis homologs are reported to the chloroplast. We then leveraged a recently certified high-throughput fluorescent tagging strategy to obtain subcellular localizations for 47 proteins in Chlamydomonas. Results yield the initial experimental report of 98% of these proteins in Chlamydomonas, including 12 chloroplast localizations. We use these to perform analysis and speculate on possible pathway roles. We propose one gene as a γ-glutyl-cyclo-transferase and offer future directions. Proposals aim to inform the work needed to understand Chlamydomonas going forward.

A Novel Protocol for Guiding Research into Inter-Microbiome Horizontal Gene Transfer of Biosynthesis Gene Clusters

Christian Hernandez
Advisor: Mohamed Abou Donia

Microbial communities constantly share genes via horizontal gene transfer (HGT) and cur- rent research has focused on HGT within microbial communities and for antibiotic resistance genes (ARGs). However, microbes transfer genes outside of their microbiome and ARGs are only one gene type located within larger sequences known as biosynthesis gene clusters (BGCs). Unlike ARGs, existing research lacks a framework for identifying in what environments BGCs are likely to be transferred across microbiomes. We conducted an investigation to identify BGC-HGT events and environments favorable for HGT between soil and human microbiomes. We began by comparing BGCs in soil and human stool metagenomes from the same geographic location. Results indicated instances of shared BGCs but a very low HGT rate. We realized we needed to identify definite BGT-HGT hotspots, which we named transsynvirons, before searching for BGCs shared across the samples. By reviewing the literature on HGT, ARGs, and BGCs, we identified agricultural, floodplain, and coastal regions as likely hotspots for BGC-HGT. These transsynvirons were quantitatively defined using internationally recognized definitions of habitats. Then, we designed a processing protocol to input stool metagenome samples, identify their ecoregion, match them to soil samples, and use the definitions to certify or deny sample collection sites as transsynvirons. The protocol matched and processed 565 human stool samples and 67 soil samples across five ecoregions. Differing national definitions of transsynvirons were resolved, and the Hu- man and Terrestrial Metagenome Databases provided compatible soil and stool samples. In sum, the study advanced the field of inter-microbiome genetics, addressing gaps like site selection for metagenome

sampling and the importance of uploading metagenome data into geography-based databases. We also designed a protocol to be adopted broadly in the microbiome genomics community. Future work includes running datasets filtered by the protocol through anti-SMASH to identify BGC-HGT events and adjusting the protocol to include other transsynvirons.

Identifying Potential Molecular Mimics of Orexin Peptides and Receptors in Support of a T cell- Mediated Autoimmune Basis for Narcolepsy Type 1

Haley Hoffman
Advisor: Lisa Boulanger

Narcolepsy is a neurological sleep disorder with a biological basis that is characterized by excessive daytime sleepiness (EDS), cataplexy (type 1 only), hypnagogic hallucinations, and abnormal sleep cycles. Narcolepsy type 1 (NT1) is caused by the selective destruction of orexin(hypocretin)-producing neurons in the lateral hypothalamus. Orexin in a neurotransmitter that is essential for maintaining arousal, wakefulness, and appetite. The mechanism of targeted neuronal cell death is unknown, but the literature has created a hypothesis of T cell-mediated autoimmunity caused by molecular mimicry. The genetic predispositions of the MHC class II molecule (DQ0602), T cell receptor D (TCRD), and others are not a sufficient cause but combination with an environmental trigger (Influenza A, Streptococcus pyogenes, Pandemrix 2009 H1N1 Vaccine) could mediate the attack. This thesis focuses on finding amino acid sequence homology between orexin, its receptors, and infectious pathogens of humans using blastp to begin to identify peptides with mimicry to host peptides and potential to be presented by MHC class II on antigen presenting cells for TCR targeting. The results show peptide mimicry with many protein epitopes from Influenza A and B, Streptococcus pyogenes, and many bacterial species. This experiment is the first step in identify peptides with sequence homology for further characterization to find structural homology and mimicry.

Investigating the ACAT2/GP78/INSIG1 Tri-Protein Complex

Chloe Holland
Advisor: Nieng Yan

The ER membrane protein ACAT2 is responsible for converting free cholesterol into storable cholesteryl ester. Increased ACAT activity and the subsequent buildup of esters is a major contributor to hypercholesterolemia and cholesterol-induced diseases, including atherosclerosis and several forms of cancer. ACAT2 is a promising drug target to counter cholesterol-associated diseases because its activation is inducible and it is selectively localized in only two cell types: the enterocytes in the intestines and the hepatocytes in the liver. Recent findings have identified that the proteins INSIG1 and GP78 facilitate the ubiquitination and subsequent degradation of ACAT2 via the ubiquitin proteasome pathway. It is hypothesized that during this process ACAT2, INSIG1 and GP78 form a tri-protein complex wherein INSIG1 is directly bound to both ACAT2 and GP78, and ACAT2 and GP78 are not bound to each other. Thus far, no cryo-EM structure has been resolved for GP78 or INSIG1 alone, nor for the entire ACAT2/GP78/INSIG1 complex. GP78 and INSIG1 also facilitate the degradation of other key proteins in the cholesterol pathway, including the protein HMGCR, which catalyzes the rate-limiting step of cholesterol biosynthesis. Resolving the structure of the ACAT2/GP78/INSIG1 complex could reveal crucial information about the mechanisms of GP78 and INSIG1-mediated ubiquitination in general, and could pave the way for the development of new therapies for treating hypercholesterolemia. To lay the groundwork for the elucidation of this challenging structure, ACAT2, GP78 and INSIG1 plasmids were generated, purified and transfected into HEK293F suspension cells. Cells were harvested and proteins were collected and purified. Protein purification was optimized by changing several parameters, including the concentration of detergent and construct of protein selected to purify (full length vs dNTD). A Western Blot was performed to test for the presence of ACAT2 and GP78. Cryo-EM samples were prepared and preliminary structural data was collected. Here, we present preliminary findings

including agarose gels to verify plasmid expression, SEC chromatograms of different optimization conditions, SDS-Page gels of purified proteins, Western Blots testing for ACAT2 and GP78, and Cryo-EM images. We hope that our purification troubleshooting of the three proteins and optimized methodology will lead to a resolved ACAT2/GP78/INSIG1 cryo-EM structure.

CXCL12-CXCR7 SIGNALING AXIS REGULATES MAMMARY STEM CELLS IN NORMAL MAMMARY GLAND DEVELOPMENT

Jason Hong
Advisor: Yibin Kang

Mammary stem cells (MaSCs) reside at the top of the differentiation hierarchy within the mammary epithelium and are key drivers of mammary gland development. Although MaSCs share transcriptional regulators and differentiation mechanisms with breast cancer stem cells (BCSCs), the microenvironmental factors governing their regulation are still unclear. Previous literature has implicated the importance of paracrine signaling between the basal and luminal mammary epithelial layers in promoting mammary gland morphogenesis, but there is a lack of studies investigating the exact signaling molecules and comprehensive mechanisms involved. Therefore, this study examines the role of homeostatic chemokines in affecting mammary gland development and MaSC activity. Through microarray gene expression data, we identified luminal-expressing chemokine ligand, CXCL12, and its basal-expressing chemokine receptor, CXCR7. After validating the protein expression of these chemokines, we investigated their functions through the generation of luminal-specific CXCL12 and basal-specific CXCR7 knockout mice. Our results ultimately demonstrate the importance of the CXCL12-CXCR7 signaling axis in promoting ductal branching morphogenesis, mammary epithelial cell proliferation, and MaSC activity. Furthermore, we found evidence for the existence of bidirectional crosstalk between the luminal and basal layers, once again mediated by signaling between CXCL12 and CXCR7. In addition to highlighting a novel role for CXCL12 and CXCR7 in regulating MaSCs during mammary gland development, our results have future implications in the initiation of breast cancer tumorigenesis by BCSCs. This study will thus provide a foundation for the future identification of homeostatic chemokines as new therapeutic targets for breast cancer therapy.

Steps Toward a New Biological Canon: Directed Evolution of a Structurally Unique *de novo* ATPase

Kaelix Johnson
Advisor: Michael Hecht

Throughout time evolution has proven to be a powerful tool for advancements in biology. From these advancements come patterns in form and function for both effector molecules like proteins and substrates like nucleoside triphosphates (NTPs). NTP binding itself is one of the earliest identified binding motifs in naturally occurring proteins with one binding motif, the Rossmann fold (an $\alpha\beta\alpha$ fold dubbed the $\alpha\beta\alpha$ sandwich), present within a sizable portion of all known proteins found in the Protein Data Bank. Associated with the Rossmann fold is the phosphate loop (P-loop) motif (Consensus: GXXXXGK[ST]), a motif found in all naturally occurring ATPases. Alternative ATPase (AltTPase) is a de novo, 4-helix bundle ATPase discovered by Wang and Hecht that exhibits no characteristic $\alpha\beta\alpha$ fold commonly found in NTP binding proteins nor the presence of any P-loop. However, AltTPase shows poor catalytic ability when compared to naturally occurring enzymes. We demonstrate that this catalytic rate can be improved qualitatively through directed evolution with selection done on a life-or-death basis

with survival being linked to improved ability. Despite undergoing evolution and increasing in catalytic ability, S7-55 still shows no $\alpha\beta\alpha$ fold, nor any β-sheet identity either. Furthermore, S7-55 still exhibits no signs of evolving a traditional P-loop motif.

Investigating Ribonuclease L's Cleavage Bias Towards Uracil: A Retrotransposon Perspective

Chris Jun
Advisor: Alexei Korennykh

The OAS/RNase L pathway plays an instrumental role in antiviral defense by recognizing viral double-stranded RNA. However, recent work in the field has shown that RNase L plays a role in other homeostatic roles in the cell, such as recognition of endogenous cellular double-stranded RNA molecules. Preliminary findings in our lab have showcased that endogenous double-stranded RNA may originate from intronic inverted pairs of retroelements. Furthermore, introns encoding for endogenous double-stranded RNA are also enriched for uracil. Thus, my thesis investigated the potential connections between intronic uracil enrichment and retroelement insertion into the genome. Utilizing custom-built programs that extracted nucleotide sequences of retroelements in the human genome, I observed that L1 and Alu retrotransposons and their flanking sequences are enriched for adenine. Moreover, I also found that intronic L1/Alu insertion are majorly found in the antisense direction. Given these findings, I propose two potential models in which L1/Alu antisense-biased integration into the human genome can occur. Both hypothesized mechanisms provide explanations for our lab's previous findings of enriched uracil in introns containing higher retroelement insertions which form endogenous dsRNA, and thus provides molecular clues into RNase L's long observed and unclear UN^N cleavage consensus site. Therefore, my senior thesis serves to propose novel homeostatic functions of the OAS/RNase L system as well as helps to address critical gaps of knowledge regarding this innate immune system pathway.

Elucidating the Dynamics of Pyrenoid Biogenesis Through Intergenic Suppression of saga1

Angelo Kayser-Browne
Advisor: Martin Jonikas

The enzyme Rubisco drives the fixation of atmospheric carbon into organic compounds, and the growth of photoautotrophic organisms is inherently dependent on Rubisco-CO2 kinetics. Algae provide Rubisco with concentrated CO2 at the pyrenoid, an organelle within the chloroplast which drives one-third of global carbon fixation. The biogeochemical significance of the pyrenoid makes it a promising bioengineering target, necessitating an understanding of the genes involved in pyrenoid biogenesis. In the alga Chlamydomonas reinhardtii, the gene SAGA1 is required for normal growth and pyrenoid formation, and knockout leads to severely limited growth in air and the formation of multiple pyrenoids with key structural defects. The multifaceted nature of this mutant phenotype suggests that numerous genes may be misregulated in the absence of SAGA1, collectively leading to the observed changes in pyrenoid morphology and growth. However, the identities of these genes and their relationships to SAGA1 are largely unknown. We performed a suppressor screen to determine if the knockout of certain genes could rescue the saga1 mutant. A subset of suppressor mutants showed partial recovery of wild-type features, including improvements in pyrenoid morphology and the ability to grow in air. Our efforts provide quantitative phenotypic information on these mutants and lend credence to a model in which canonical pyrenoid formation is at least partly dependent on the presence of a tubule network and precise phosphorylation of the linker protein EPYC1. Given the implications of pyrenoid formation on carbon fixation as a whole, these results represent a crucial step toward the realization of bioengineered CCMs in crops to address burgeoning concerns in agriculture and climatology.

Optimizing AAV-Mediated Gene Delivery in the Brain: Blocking TLR9 Minimizes the Negative Impacts of AAV on Neuronal Morphology

Neerav Kumar
Advisor: Lisa Boulanger

Recombinant adeno-associated viruses (rAAVs) have transformed the fields of basic neuroscience and human gene therapy. Although rAAVs are considered relatively non-immunogenic, AAV elicits an immune response in the brain, including upregulation of immune proteins known to negatively regulate dendritic complexity and synaptic transmission. This suggests that AAV could alter the structure and function of neurons, a previously unexplored possibility. To quantify AAV-induced changes in neuronal morphology, DiOlistic labeling, Sholl analysis, and Strahler analysis were performed on layer 4-6 pyramidal cells in mouse primary somatosensory cortex. We found that AAV simplifies dendritic morphology, irrespective of AAV serotype, promoter, transgene, or production facility. We demonstrated that AAV induces a broad spatial reduction in dendritic complexity by 21 days post injection, which is associated with a decrease in cortical cell density. By corroborating electrophysiology recordings with Western blot quantification, we determined that AAV changes synaptic transmission by altering the ratio of Ca2+-permeable to Ca2+-impermeable AMPA receptors. To determine if the toll-like receptor (TLR9), which detects viral DNA, is required for AAV detection in the brain, TLR9 was inhibited. We found that systemically blocking TLR9 prevents AAVinduced dendritic simplification. In this thesis, for the first time, we characterize the negative impacts of AAV on neuronal morphology. We propose to block TLR9 as a solution to minimize these unintended consequences of AAV in basic neuroscience research and to develop safer and more effective gene therapies.

"I Want to Get Better:" An Inquiry into Current Psychopharmacological Treatment Protocols of Major Depressive Disorder

Annick La-Branche
Advisor: Jeffry Stock

Major Depressive Disorder (MDD) afflicts a proportion of the global population. Nevertheless, its cause has proven elusive. As a result of the first use of antidepressant psychopharmacology in 1951, the neurochemical imbalance, or monoamine, theory of depression emerged and continues to be the foundation of current understanding of MDD even though the evidence does not support this theory.1,2 Antidepressants, particularly Selective Serotonin Reuptake Inhibitors (SSRI) and Serotonin-Norepineprhine Reuptake Inhibitors (SNRI), are the focus of this work. Their development and use are traced alongside the evolution of current views regarding MDD. Through the use of a comprehensive literature review informed by multiple disciplines and empirical evidence, an analysis of current practice was undertaken. This analysis concludes by highlighting the need for a rethinking of MDD and posing questions that may serve to reëvaluate how one interacts with ideas of mental illness, treatment, and healing as a society and as an individual.

Investigating the Regulation of Desmosomes During Mouse Hair Follicle Morphogenesis

Tori Laurencin
Advisor: Danelle Devenport

Convergent extension, or the simultaneous shortening and elongation of tissue along orthogonal axes, is a process that regularly occurs during embryogenesis. Convergent extension has been predominantly studied in Drosophila, where it was discovered that the underlying mechanism behind the process in epithelial cells is cell intercalation, or the exchanging of neighboring cells. Currently, the leading model to describe cell intercalation centers on the

rearrangement of cell-cell junctions called adherens junctions. However, there are other types of junctions that exist that the current leading model does not address. For example, unlike in the Drosophila, the mammalian epidermis is rich with another type of cell junction: desmosomes. Desmosomes link the intermediate filament cytoskeleton of adjacent cells. They enable the epidermis to withstand mechanical forces and serve as particularly strong adhesion between cells. Considering their particularly "sticky" nature, hypothetically, how would desmosomes remodel during cell intercalation in mammalian skin epithelial cells? Here, I report the findings of my project investigating how desmosome remodeling occurs during cell rearrangement. A specific process in mammalian skin development I am studying to further understand desmosome remodeling during cell rearrangement is hair follicle morphogenesis. Hair follicles develop from hair placodes, clusters of epithelial cells that arise from an initially undifferentiated epidermis. After forming, hair placodes bud downward into the dermis and later become polarized through cell intercalation so that they point and grow in an anterior direction. Whole-mount immunostaining and correlating fluorescence intensity heat maps showed that expression of desmosomal proteins desmoplakin and plakoglobin are lower in the hair placode relative to unspecified interfollicular cells. Subsequently, I investigated whether or not planar cell polarity (PCP), a developmental cue that sparks hair placode cell rearrangement, was the trigger for desmosome remodeling. Whole-mount immunostaining and correlating fluorescence intensity heat maps looking at both wild type and PCP mutant skin showed that expression of desmosomal proteins desmoplakin and plakoglobin remained lower in the hair placode relative to unspecified interfollicular cells. Taken all together, this data suggested that decreased desmosomal proteins in hair placodes allow for hair placode cell movement to occur. PCP, however, is not a developmental cue that triggers desmosome remodeling. In the future, one should characterize the expression of other desmosomal proteins and their localization. Other possible developmental cues, such as the the Wnt/Beta-catenin pathway and the Shh signaling pathway, should also be explored to see if they trigger desmosomal remodeling.

Mutational Analysis of the Putative Lipid Transfer Protein YhdP in Escherichia coli

Michael Lee
Advisor: Thomas Silhavy

The outer membrane (OM) of Gram-negative bacteria is an asymmetric bilayer–with lipopolysaccharide molecules in the outer leaflet and glycerophospholipids (PLs) in the inner leaflet–that confers antibiotic resistance. However, how PLs are transported from the inner membrane (IM) to the OM for assembly is poorly understood. To help elucidate this process, we investigated a protein in the IM, YhdP, that is hypothesized to act as a bridge that transport PLs from the IM to the OM. Using the Phyre2 structural prediction server, we identified regions of YhdP that are homologous to predicted lipid transport proteins (LTPs). We also discovered a CxxC motif, which is usually involved in redox functions and is found in redox enzymes, in YhdP. In order to explore YhdP function and examine whether this IM protein behaves like a bridge for intermembrane PL movement, we successfully generated loss-of-function mutations in the LTPlike domains, the transmembrane domain, and the CxxC motif of YhdP. We then screened for suppressors of these mutations and found that two mutations in the LTP-like domains are suppressed by decreasing the activity of MlaA. MlaA is an OM lipoprotein component of the Mla system, which functions to maintain the asymmetrical structure of the OM. Our data demonstrate that compromising the Mla system suppresses the defects of our yhdP mutations and supports a model where YhdP facilitates the transport of PLs to the OM from the IM.

IMPACT OF AGE ON EFFICACY OUTCOME MEASURES IN PROGRESSIVE MONOGENIC DISEASES TREATED WITH AAV GENE THERAPY

Cameron Levy
Advisor: S. Jane Flint

Gene replacement therapy is a rapidly growing therapeutic modality that is uniquely suited to address monogenic diseases. Many monogenic diseases afflicting the nervous and sensory nervous systems present with a progressive pathology due to death of long-lived cell types that do not replenish quickly. Due to rapid disease progression, early intervention can prevent irreversible damage and improve patient outcomes. The present study investigates AAV gene therapy clinical trial results from six monogenic diseases (Spinal Muscular Atrophy, Leber Congenital Aumorosis, Aromatic l-amino Acid Decarboxylase Deficiency, Leber Hereditary Optic Neuropathy, Choroideremia, and Retinitis Pigmentosa) to determine whether age of intervention has a significant effect on patient outcomes. The study demonstrated correlation between younger age and improved efficacy of gene therapy in three disease that met the inclusion criteria. These results support expansion of universal newborn screening protocols to include some, if not all, of the diseases investigated. Additionally, these data highlight the potential benefit of conducting clinical trials in younger patients for progressive monogenic diseases with gene therapies coming through the clinical pipeline.

Regulation of Age-Related Cognitive Decline by the C. elegans Adult Neuronal IIS/FOXO Transcriptome

Sarah Lin
Advisor: Coleen Murphy

Elucidating the molecular factors and signaling pathways responsible for age-related cognitive decline remains one of the most important goals in the aging field. The insulin/insulin-like growth factor-1 signaling (IIS) pathway has been implicated in cellular activities ranging from longevity to stress response and is conserved from C. elegans to mammals. Previous work has found that mutation of the IIS receptor homolog DAF-2 doubles the lifespan and extends the memory capabilities of C. elegans. These longevity phenotypes are dependent on the activity of DAF-16, a forkhead box O (FOXO) protein transcription factor. While the Murphy Lab has identified the entire suite of neuronal IIS/FOXO targets in both daf-2 and daf-2;daf-16 young adult worms, the neuronal IIS/FOXO targets in aged worms have yet to be characterized. Neuronal IIS/FOXO targets in aged worms are of particular interest as they may reveal candidate genes that can slow down cognitive decline with age. This thesis investigates the differential gene expression between aged daf-2 and daf-2;daf-16 neuronal transcriptomes in order to identify and validate candidate genes that may be responsible for extended learning and short/intermediate-term associative memory in aged daf-2 worms. We show that genes dod-24 and F08H9.4 are involved in aged daf-2 mutants' ability to learn; furthermore, genes alh-2, mtl-1, and C44B7.5 may be responsible for aged daf-2 mutants' maintained intermediate-term associative memory. By identifying candidate genes potentially responsible for improved cognitive abilities with age, this study contributes to the field's understanding of the regulation of age-related cognitive decline by the IIS pathway and provides potential therapeutic targets for maintaining cognitive abilities with age.

Characterizing the role T-cell MTDH in regulating T-cell exhaustion and driving pro-tumor immunity in breast and colorectal cancer

Chythanya Murali
Advisor: Yibin Kang

Many cancer immunotherapies like immune checkpoint blockade and adoptive cell therapies aim to boost anti-tumor mediated-immunity vía reversal of T-cell dysfunction or exhaustion. However, the efficacy and use of these therapies are limited by primary or evolved resistance—phenomena that are not well-understood mechanistically. Previous studies have found that whole-body knockout of Metadherin (MTDH)—an oncogene overexpressed in several different types of cancer—reduces expression of exhaustion markers and improves infiltration of tumor-infiltrating T-cells. Additional preliminary evidence from CD4+ and CD8+ antibody depletion studies in our lab suggest T-cells to be key drivers of tumor suppression in MTDH knockout (KO) mice; however, it remains unclear as to whether T-cell MTDH regulates T-cell exhaustion or recruitment to promote pro-tumor immunity. Despite finding increased killing of PY8119 mammary and MC-38 colorectal (CRC) cancer cells by ex-vivo activated MTDH KO OT-1 cells pointing towards intrinsic immunosuppression by MTDH, we were not able to validate these findings in MTDH-/-Rag1-/- mice upon transfer of these activated CTLs. Furthermore, non-significant reductions in tumor growth upon MTDH WT recipient mice with KO bone marrow allografts, initially indicating a non-significant role for immune and T-cell MTDH in modulating T-cell effector activity. However, in MTDH KO recipients transplanted with WT bone marrow, significant rescue of tumor growth is observed contradicting earlier findings. Although further validation of our co-culture and murine models is needed, these findings suggest a role of whole-body MTDH KO in molding anti-tumor immunity. Our findings overall point to a seemingly complicated and interdependent network of extrinsic versus intrinsic modes of MTDH-mediated T-cell regulation that requires further elucidation. Grasping the full scope of MTDH-mediated T-cell regulation will be critical to optimizing the safety and efficacy of MTDH-based therapeutics.

Contextualizing the Contributions of HP1α-Mediated Chromatin Cross-Linking to Total and Internal Nuclear Mechanics

Natalia Orlovsky
Advisor: Clifford Brangwynne

The physical properties of the nucleus dictate global cell responses to mechanical challenges, and their disruption is implicated in human disease. For instance, nuclear softening facilitates the rate-limiting deformation step in cancer cell invasion. Recent work suggests that chromatin-chromatin cross-linking is an important (but under-explored) determinant of nuclear mechanical properties. Here, I examine whether depletion of HP1α, a chromatin cross-linker whose expression is lost in metastatic cells, changes total and internal nuclear mechanics in ways that facilitate confined cell migration. Additionally, I aim to contextualize the scope of HP1α's mechanical contributions by comparing them to those of a second, better-characterized determinant of nuclear stiffness (Lamin A). Using a set of imaging-based readouts, I demonstrate that HP1α contributes to nuclear morphology and nucleoplasmic diffusion dynamics, but that its effects are comparatively subtle. However, I also show that HP1α is a major contributor to intra-nucleolar diffusion dynamics, meaning that its impacts on internal nuclear mechanics exhibit an intriguing spatial heterogeneity. Using atomic force microscopy, a custom nuclear deformation assay, and an in vitro assessment of confined migration frequency, I then demonstrate that loss of HP1α-mediated cross-linking yields total nuclear softening and facilitates migration through narrow pores. Collectively, these results suggest an interesting comparative model of Lamin A and HP1α's contributions to nuclear mechanics and demonstrate that spatially heterogenous changes in internal mechanical properties can result in total nuclear softening. In addition, the presented findings indicate that a mechanical model of HP1α's contributions to cancer cell migration – and, by extension, to disease progression – is worth exploring further in future work.

A Farewell to Arms: Breaking the Species Barrier of Hepatitis B Virus in Small Non-human Primates

Debby Park
Advisor: Alexander Ploss

Hepatitis B virus (HBV) remains a major cause of liver disease in humans, affecting approximately 257 million people worldwide. Despite the availability of treatments for chronic HBV, a cure remains elusive. As novel therapeutics to combat HBV persistence are being developed, animal models are urgently needed to evaluate their efficacy and safety. The narrow host tropism of HBV remains a challenge in establishing a long-lasting HBV infection model in vivo, but recent studies have provided optimism toward a small nonhuman primate (NHP) model. Previous research has shown that HBV restriction in NHP species is primarily attributed to incompatibilities between the preS1 region of the viral envelope protein and the cellular functional receptor – sodium taurocholate co-transporting peptide (NTCP) – in non-permissive primates. Particularly, amino acids 157-165 in Old World monkey (OWM) NTCPs appear to be incompatible with the viral envelope. NTCP from the cynomolgus macaque (CynoNTCP), an OWM, was manipulated in this study to investigate the mechanism of HBV restriction in OWM at the entry level. Since the humanizing substitution R158G on CynoNTCP has been found to be necessary for HBV binding and uptake when expressed on HepG2 cells, CynoNTCP variants with humanized mutations in combination with the R158 mutation were constructed as follows: G157K, I160V, L161I, and P165L. To investigate the role of these residues in virion uptake, HBV preS1 peptides conjugated with FITC fluorophore were used for imaging, and flow cytometry to determine the binding ability of CynoNTCP mutants. Additionally, an HBV infection assay was conducted on HepG2 cell lines expressing the above CynoNTCP variants. The results revealed that the G157K & R158G variant reduced the ability of CynoNTCP to mediate binding and uptake of HBV virions, whereas the R158G & P165L variant enhanced infection levels significantly. This suggests that 157K may play negative role in HBV infection, while 165L is another important residue in NTCP that can confer or enhance HBV susceptibility. This novel information sheds insight into the evolutionary strategies of HBV resistance that were established during the emergence of OWM, and it may aid future studies that aim to expand HBV tropism and build a small NHP model to bolster tools for studying HBV in vivo.

Signal Transduction Architectures Underlying Population-Scale Decisions in Vibrio cholerae

Joseph Prentice
Advisor: Bonnie Bassler

Biofilms are communities of bacterial cells engulfed in a self-produced matrix that protects resident cells from threats, including antibiotics, shear stress, and predation. Over the past two decades, the intracellular and intercellular signaling systems that control the biofilm lifecycle have been revealed. In many species of bacteria, including the global pathogen and model biofilm former Vibrio cholerae, two core signaling systems play overarching roles in driving bacterial populations to form biofilms, and these systems dictate biofilm architectural features. First, quorum sensing, a mechanism of cell density-dependent chemical communication that orchestrates collective behaviors. Second, the second messenger c-di-GMP molecule that modulates effectors to control gene expression. In V. cholerae, high levels of c-di-GMP promote biofilm formation, while high cell density quorum-sensing signaling inhibits biofilm formation. The goal of my thesis was to define how biofilm formation and dispersal are shaped by the integration of c-di-GMP and quorum-sensing signaling in V. cholerae. I developed an experimental system that enables precise modulation of the activity of a V. cholerae c-di-GMP-metabolizing enzyme and I characterized its signaling properties. I chose the NspS-MbaA system, which detects the polyamines spermidine and norspermidine. Detection of spermidine by NspS-MbaA inhibits

biofilm formation via MbaA-mediated c-di-GMP degradation, while detection of norspermidine promotes biofilm formation via MbaA-mediated c-di-GMP production. I showed that the system is highly sensitive to polyamines and that the c-di-GMP produced or degraded by MbaA has an exquisitely specific effect on biofilm gene expression. Thus, although MbaA appears to transmit c-di-GMP information locally to a biofilm gene-specific effector, I also showed that alterations in the global intracellular c-di-GMP pool can elicit biofilm-specific transcriptional changes. Using the NspS-MbaA system and ligands for quorum-sensing receptors, I modulated c-di-GMP signaling and quorum-sensing signaling simultaneously. Contrary to expectation, I discovered that the high cell density quorum-sensing signaling regime enhanced polyamine-mediated biofilm formation. I found that the synergy between polyamine signaling and quorum-sensing signaling is a consequence of upregulation of the operon containing nspS and mbaA that occurs in the high cell density quorum-sensing signaling regime. Higher levels of NspS and MbaA enhance the sensitivity the signal relay to changes in polyamine concentrations, and thus, intracellular c-di- GMP levels are significantly elevated in the high cell density and high norspermidine signaling regime. I further characterized the mechanism by which elevated intracellular c-di-GMP overcomes quorum-sensing mediated repression of genes encoding biofilm structural genes. I propose that quorum sensing confers plasticity to biofilm morphogenesis by altering the levels of c-di-GMP-metabolizing enzymes as the bacterial population grows. In particular, by increasing NpsS-MbaA levels over the course of growth, the bacterial population delays commitment to norspermidine-mediated biofilm formation until members of the community detect one another's presence via quorum sensing. Further tests of this model of quorum-sensing and c-di-GMP signal integration for other c-di-GMP-metabolizing receptors would clarify its generality. Going forward, probing the interactions between quorum-sensing and c-di-GMP signaling in species that occupy diverse niches and that have lifestyles that differ dramatically from that of V. cholerae could deliver a unified picture of how the coordination of sensory signaling systems is linked to the ecological and evolutionary roles that biofilms play across the bacterial domain.

The Characterization of the Pin1 Homolog, Dodo, in Drosophila Melanogaster

Paulina Przygonska
Advisor: Daniel Notterman

Although traumatic brain injury (TBI) is associated with neurodegenerative disorders, the mechanisms underlying the association remain unclear. To study potential mediators in these mechanisms, Drosophila melanogaster has been increasingly used. Tau, a protein that mediates axonal transport and microtubule stabilization within the brain, becomes hyperphosphorylated, mislocalized, and forms aggregations in neurodegenerative disease. However, tau and its mediators have not been studied in the context of TBI-induced neurodegeneration in Drosophila. Though, Pin1, a prolyl isomerase, has been shown to isomerize tau and prevent tau-induced cellular toxicity in both traumatic brain injury and neurodegeneration, it remains unknown whether Dodo, the Drosophila homolog of Pin1, is also able to isomerize tau. Increasing evidence suggests that Pin-1 may induce apoptosis in neurodegenerative disease and that mitochondrial fission may mediate this Pin1-induced apoptosis. Thus, the presence study attempts to characterize the function of Dodo in Drosophila and its downstream effects on mitochondrial dynamics. Our work demonstrates that Dodo mutant flies exhibit significant decreases in median lifespan, trending decreases in mitochondrial DNA copy number and alterations in mitochondrial fission and fusion gene expression. Our work also shows vacuolization is present in the Drosophila Dodo

mutant flies as soon as 9 days after eclosion. Our work suggests that Dodo plays a role in mediating mitochondrial dynamics, and its disruption may promote neurodegeneration. Therefore, the Dodo protein may be a suitable target for further investigation within the pathways underlying neurodegenerative disorders.

History is in Our Blood: Systemic Sclerosis in the Oklahoma Choctaw

Brooke Redwine
Advisor: Jeffry Stock

Systemic sclerosis is a rare connective tissue disease characterized by skin hardening, damage to vasculature, autoimmunity, and internal organ involvement. A high prevalence of systemic sclerosis has been reported in the Oklahoma Choctaw. Unfortunately, the only reported prevalence comes from Arnett et al.'s 1996 study: much is unknown about the disease in the Choctaw today. For the present research, data was obtained from the Talihina Service Unit of the Choctaw Nation to calculate an updated prevalence of systemic sclerosis in the Choctaw. Analyses on the nature of the disease and its related diagnoses in the Choctaw were also investigated. The prevalence of systemic sclerosis in the Choctaw was calculated to be 63 per 100,000 (95% CI: 39 – 96), not statistically different from the 1996 result of 66 per 100,000 ($p = 0.88$). The prevalence in non-Choctaw Native Americans was 27 per 100,000 (95% CI: 3 – 638). For full-blood Choctaw, the prevalence was 115 per 100,000 (95% CI: 6 – 80). The prevalence of systemic sclerosis in the Choctaw was found to be significantly different than that of the U.S. general population (odds ratio = 2.58, 95% CI: 1.63 - 3.86, $p = 0.00013$). These updated calculations strengthen previous indications that the prevalence of systemic sclerosis in the Oklahoma Choctaw is atypically high. This research also offers insights into the various symptoms of systemic sclerosis by investigating their possible relationships to the disease and providing analyses of their nature among the Choctaw.

Investigating the sorting and intracellular transport of planar cell polarity proteins Frizzled6 and Vangl2 to and from the cell junction

Daisy Rodriguez
Advisor: Danelle Devenport

Planar cell polarity (PCP) is an essential feature that describes the coordinated orientation of cell polarity across a tissue and has been implicated in mechanisms across different cell types and cell tissues. PCP is established through the asymmetric distribution of PCP components within cells, and which share interaction with PCP components on adjacent cell, creating a system for cell-to-cell communication of polarity across tissue. Disruptions in the genes which transcribe the PCP components lead to loss of uniform PCP in tissue as well as effects in development of organs, the skeletal system, and during embryogenesis. Because of its importance in development of a variety of different tissues and its implications in human health and disease, it is important to understand the mechanism for the PCP pathway, specifically how the asymmetrical localization of PCP core proteins is established. Previous studies have focused on the intracellular trafficking of core PCP proteins and found that the Frizzled6 (Fzd6) and Vangl2 proteins exit the Golgi in separate compartments guided by sorting motifs that are part of different mechanisms. Though the sorting motifs were found to be necessary for this differential compartmentalization, the question of whether they are sufficient for this was not been answered. The investigation of this differential exit of the Golgi in a polarized system like the mammalian skin also offers the ability to ask questions about its implications in asymmetric localization The mode of trajectory to and from the cell junctions is also important to investigate in an attempt to better characterize trafficking of Fzd6 and Vangl2 in

the mammalian skin. The goals were to use a protein-sequestering assay to halt protein trafficking and be able to visually see how vesicle colocalize.

Dancer's high: an experiential review of cannabinoid activity and improvisational street dance

Juan Sarmiento
Advisor: Jeffry Stock

Dance performances in contemporary social settings are irremediably associated with substance use. From electronic music festivals to nightclubs and small gatherings, marijuana appears to be the primary psychoactive consumed among casual and professional dancers alike. Though some research has explored the effects of marijuana consumption on social disinhibition and creativity, these studies were conducted in laboratory settings divorced from the real-world scenarios in which creators consume Cannabis products while engaging in their craft. Indeed, the vast amount of available literature in the cannabinoid field is concerned with medicinal applications, generally overlooking the potentially valuable information that observational studies on popular marijuana usage can provide on the physiological effects of the drug. Desiring to delve into the interactions that Cannabis dosing has on dance as a creative expression, several career-level artists participating in Street Dance communities were interviewed on their exposure to and subjective experiences with marijuana and dance. Critical analysis of this experiential data indicated significant overlap in the mental and physical effects felt while being 'in the zone' to existing research on cannabinoid signaling, most relevant of which is the 'runner's high' phenomenon recently shown to link physical activity with analgesia and euphoria through regulation of endocannabinoids. A corresponding 'dancer's high' is proposed to portray how stimulation by Cannabis may lead to a self-sustaining state of increased creativity for dancers.

Characterizing the Effect of Quorum Sensing on Aggregation and Virulence in Pseudomonas aeruginosa

Bob Schofner
Advisor: Bonnie Bassler

Quorum sensing (QS) is a cell-to-cell communication process performed by bacteria, including human pathogen Pseudomonas aeruginosa, to coordinate the expression of virulence genes through the secretion and reception of a common signal molecule, termed an autoinducer. In addition to the characterized P. aeruginosa specific QS signaling pathways, surprisingly, both virulence factor expression and biofilm formation in P. aeruginosa have been reported to be regulated by a "universal" autoinducer called AI-2 through an unknown mechanism. While P. aeruginosa has not been reported to produce AI-2, this universal autoinducer is synthesized by hundreds of species of bacteria and is often associated with chemotaxis and biofilm formation. To experimentally determine AI-2's effect on P. aeruginosa's aggregation and virulence, brightfield imaging was used to visualize aggregate formation with varying concentrations of AI-2. Additionally, to investigate the influence of specific chemotaxis genes on aggregation, PAO1 mutagenesis was utilized to generate the P. aeruginosa mutants ΔcheY and Δpsl. Brightfield and stain-based florescence imaging were then used to quantify the aggregation phenotype of each mutant. While AI-2 was shown to have no observable influence on P. aeruginosa aggregation, the knockout mutant aggregation quantification assays showed that the quorum sensing and chemotaxis systems have clear relevance. While AI-2 may not be a novel therapeutic for Cystic Fibrosis patients with chronic Pseudomonas aeruginosa infections, there still exists the potential for other mechanisms of aggregate formation and dispersal to overlap with QS and be targeted therapeutically in the future.

Designing a Framework for Investigating the Role of Active Transcription, Mismatch Repair and Chromatin Accessibility in Prime Editing

Anmoldeep Singh
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Although CRISPR/Cas systems have revolutionized gene editing, their applications in clinical therapies and research are still limited by their reliance on double-strand breaks (DSBs). Prime editing (PE) is a new technology which utilizes a Cas9 nickase fused to a reverse transcriptase to directly transcribe small, precise edits at target sites, foregoing the need for DSBs. Although this technique is accurate in introducing small edits into the genome, it is still variable in its editing efficiency and a better understanding of the factors which may control these PE efficiencies is needed. In this project, I first tested the role of active transcription around the edit site, finding that modulating transcription around a reporter edit site did not impact edit efficiency of PE. As mismatch repair (MMR) had previously been implicated in affecting PE, I knocked down the MMR gene MSH3 and designed pegRNAs to find edits sensitive to this knockdown. Finally, I attempted and failed to replicate CRISPRoff silencing of eGFP close to a PE target site in order to investigate the role of chromatin accessibility on PE. However, I did formulate a new design for CRISPRoff silencing that could do this reliably. While this study did not find conclusive results on the effects of active transcription, MSH3 and chromatin accessibility on PE, it did provide experimental frameworks for future studies that may do this. This future research will better inform the cellular determinants of PE efficiencies, allowing PE to be further optimized for therapeutic and research applications.

Investigating the Potential Involvement of Poorly Characterized Chlamydomonas Genes in Photosynthesis

Arthur Sirkejyan
Advisor: Martin Jonikas

Photosynthesis is central to the continuation of life on Earth, as it uses abundant solar energy to generate the vast majority of the food that is consumed. However, inefficiencies found in the photosynthetic pathways of major crops contribute to growing concerns of food shortages amidst an ever-increasing population. Enhancing our understanding of photosynthesis may help us in identifying crucial components of the pathway which in turn may be vital to improving photosynthetic efficiency in crops. In order to contribute to this understanding, here we used gene rescue to validate the involvement of novel genes that our lab recently identified as likely having roles in photosynthesis. We successfully rescued 10 poorly characterized genes, demonstrating that they are required for normal photosynthesis, and determined the localization of 2 proteins. Our results provide a framework for the further investigation of these genes and the functions of their respective proteins, providing novel avenues for additional inquiries into the specific photosynthetic pathways they may be involved in.

An Investigation of the Antibiotic Potential of ONO-RS-082 and its Unique Mechanism of Action

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The rising threat of antibiotic resistance is a pressing issue for the maintenance of modern health and medicine. Thus, the identification of novel antibiotic candidates that target multi-drug resistant bacteria with unique mechanisms of action (MoAs) is a crucial area for future research. Here, we investigate the antibiotic properties of a compound, ONO-RS-082 (ONO), which was identified by bacterial cytological

profiling as operating via a novel MoA. We found that ONO successfully inhibits the growth of clinically relevant Gram-positive bacteria with potent bactericidal activity, low levels of resistance, and low levels of mammalian cell cytotoxicity. Furthermore, we investigated ONO's unique MoA by combining proteomic and genetic assays and identified a ribosomal subunit protein (RpsP) along with three proteins involved with regulation of cell wall shape and elongation (MreC, MurAA, and FtsL) as potential targets of ONO. We additionally confirmed that ONO induces membrane permeabilization. Though we initially hypothesized that ONO might induce cell wall permeabilization as a secondary effect of targeting one of these proteins, treatment of bacteria with ONO did not produce phenotypic effects that we would expect from this MoA. Furthermore, ONO's antibiotic permeabilization abilities acted with a faster time course than other antibiotics that affect cell wall biosynthesis and was more similar to that of membrane-targeting antibiotics. Based on these results, we hypothesize that ONO primarily works to target the membrane. Future experiments should look to understand the exact mechanism of this membrane permeabilization in order to determine the future in-vivo and clinical viability of this antibiotic candidate.

Breaking the Species Barrier: Investigating the Host Proteins Involved in Human and Murine Hepatitis B Infection

Mansi Totwani
Advisor: Alexander Ploss

Hepatitis B virus (HBV) is a highly contagious pathogen that often leads to liver fibrosis, cirrhosis, and hepatocellular carcinoma (HCC), resulting in around 887,000 deaths each year. As more than 250 million people are chronically infected with HBV and there is no cure available, there is an urgent need to gain deeper insight into the molecular details of the viral life-cycle and to develop physiologically relevant platforms for studying hepatitis B in vivo. HBV exhibits a narrow species tropism, only infecting humans and great apes; thus, a mouse model with inbred of susceptibility to HBV infection would be a major asset for analyzing the complex interplay between this virus and the mammalian host. The barriers of interspecies transmission for HBV in mouse cells are incompletely understood. Prior work suggests that the viral life-cycle is blocked a step post entry but before the intranuclear replication stages. Thus, we hypothesized that nuclear import of the viral capsid and capsid disassembly are blocked in mouse cells, presumably due to the lack of human/primate specific host factor(s). To identify such factor(s), we conducted loss-of-function and gain-of-function experiments in human and murine hepatoma cells, respectively. We identified several candidates potentially implicated in HBV infection in human cell lines, including importin-α-1 (KPNA1) and 2 (KPNA2). Through confocal microscopy, we determined that these proteins are differentially localized in HBV-infected human and murine cells, with KPNA2 and importin-β (KPNB1) appearing to be restricted outside of the nucleus in murine cells upon HBV infection. We propose a mechanism for HBV nuclear entry block in murine cells due to the accumulation of karyopherins in the cytoplasm, and we identify several nucleoporins that could be implicated in the host restriction barrier that prevent mice from being infected. In an alternative, yet complementary approach, we are in the process of conducting an unbiased screen using a cDNA library of the human ORFeome, with preliminary results suggesting that expression of a small proportion of unidentified human factors increases support of HBV infection in murine cells. Further testing is required to confirm these results, but our findings contribute significantly to the search for the barriers in host tropism of this virus and the eventual development of an immunocompetent mouse model that is fully permissive to HBV infection and can facilitate testing of therapeutics for this deadly virus.

Dynamically Restructured ER-mitochondria Contacts in Herpesvirus-Driven Modulation of the Mitophagy-Metabolism Axis

Elene Tsopurashvili
Advisor: Ileana Cristea

Viruses remodel organelles to subvert host defenses and reprogram cellular functions for virus replication. This is exemplified by human cytomegalovirus (HCMV), a prevalent beta-herpesvirus with significant health burden in infants and immunocompromised patients. A hallmark of HCMV infection is the dynamic regulation of mitochondria, whereby mitochondria are highly fragmented yet, surprisingly, exhibit upregulated metabolism necessary for virus production. This poses a conundrum, as in other contexts (e.g., neurodegeneration, other virus infections), fragmentation is coupled with decreased cellular respiration and precedes targeted mitochondrial degradation via mitophagy. It is unknown how HCMV circumvents mitophagy to meet the bioenergetic demands of the replication cycle. Infection-induced mitochondrial features are reminiscent of functions controlled by ER-mitochondria membrane contact sites (MCSs), nanometer regions of apposition between organelles that are fundamental to mitochondrial health and dynamics, enabling biomolecule transfer and coordinated regulation of function. These regions are mediated by tethering proteins, such as the protein tyrosine phosphatase interacting protein 51 (PTPIP51) and vesicle associated membrane protein-associated protein B (VAPB), which have not been investigated during HCMV infection despite their essential roles in mitochondrial calcium homeostasis, ATP production, and autophagy. Here, we integrate quantitative proteomics, super-resolution microscopy, live-cell metabolic assays, and molecular virology to uncover the temporally controlled regulation of ER-mitochondria contacts during HCMV infection. Specifically, we discover that infection induces the formation of a stabilized and asymmetric ER-mitochondria contact structure, which we term mitochondria-ER encapsulations (MENCs). MENCs sequentially recruit VAPB and then PTPIP51, increasing their protein abundances and tethering interactions throughout infection. We show that PTPIP51 is required for virus production and go on to define its dynamic protein interactome, revealing its role as a temporal scaffold in the pro-viral mitochondrial remodeling. PTPIP51 recruitment to MENCs is accompanied by time-sensitive protein interactions with the mitochondrial respiratory complexes. In agreement with this finding, we discover that PTPIP51 drives virus-induced metabolic upregulation. We also find that PTPIP51 interacts with the crucial autophagy complex ATG12-ATG5-ATG16, potentially mediating suppression of autophagosome assembly around mitochondria. Altogether, our work reveals pro-viral restructuring of ER-mitochondria contacts that recruit PTPIP51 and its interactors for a temporal modulation of mitophagy and metabolism. As dysregulation of ER-mitochondria contacts is also a characteristic of neurodegenerative and metabolic disorders, our work has implications beyond HCMV infection.

Determining the Role of the XRHAMM Protein During Microtubule Nucleation and Cancer Progression

Taraje Whitfield
Advisor: Sabine Petry

Cancers progress as a result of hyper cell proliferation, and in order for cells to divide so often, they need extra cell division machinery to do so. It has recently been found that in cancer cells, the receptor for hyaluronan-mediated motility (RHAMM) is overexpressed, and that its homolog in Xenopus laevis is also involved in microtubule (MT) nucleation of spindle microtubules—a direct link to cell division since MTs segregate chromosomes before cytokinesis. Although much is known about branching microtubule nucleation in X. laevis, but the precise role that RHAMM plays in Xenopus (XRHAMM) is yet to be discovered. In this study, I seek to learn more about XRHAMM in order to apply these findings to uncover how this protein functions in cancers. To do this, I constructed plasmids containing the N- and C-terminal fragments of XRHAMM via Gibson Assembly. Furthermore, I performed a 2 L expression of XRHAMM primer dimers tagged with GFP, Strep, and Histidine, along with a 50 mL test expression of the N- and C-terminal constructs of XRHAMM. Future directions of this research may go on to purify the Full Length XRHAMM, along with TPX2, ɣ-Tubulin Ring Complex, and NEDD1, and perform biochemical assays on these

components to see if they form a complex in vitro, as previous studies have suggested from in vivo experiments. The function of XRHAMM may also be analyzed in processes such as branching microtubule (MT) nucleation with the intention of better understanding its function in cell division of human RHAMM. Insights from this project and others like it may further the field's understanding of XRHAMM, branching MT nucleation, and possible cancer therapies that might directly target XRHAMM or any of its functions or interaction partners.

Our Microscopic Neighbors: How External Factors Affect Viability and Microbial Dynamics of Bacteria in the Built Environment

Joy Xie
Advisor: Zemer Gitai

The built environment (BE) consists of human-made structures, and the bacteria of the BE can potentially affect human health because of their constant proximity to humans. This has led to increasing public concern of whether the bacteria in the BE are harmful. Therefore, previous studies have analyzed the composition of the BE microbiome and the external factors that affect its formation and dynamics to assess if bacteria in the BE can affect human health. However, there is a gap in literature in determining the viability of bacteria in the BE with culture-independent molecular methods and assessing the relative importance of external factors in affecting the dynamics of bacteria in the BE. Therefore, the objectives of this study are to systematically profile a BE and quantify bacterial viability and to determine which external factors affect the BE bacterial population the most by isolating these factors as independent variables and measuring the resulting bacterial viability. By profiling a typical home BE, we found that the home BE microbiome has low viability by using the PMA-ddPCR approach to evaluate bacterial viability and that a large fraction of the bacteria comes from the human microbiome. We also found that water content level was the primary driver of variations in viability on BE surfaces, while location was the primary driver of differences in bacterial richness. We then isolated four external factors, surface material, human interaction, relative humidity, and temperature, to exclude confounding factors and to determine the relative impact that each factor had on bacterial viability in the BE. We discovered that relative humidity and human interaction had the largest impact, temperature had moderate impact, and surface material had the least impact. This research will better inform the scientific community of the bacterial viability in the BE, and which external factors are most important in changing microbial viability. Consequently, this understanding will update our conception of the BE microbiome to better assess whether bacteria in the BE can affect human health and to take steps in maintaining a healthy microbiome indoors.