XMAP215 & TPX2: Investigating the Role of Spindle Assembly Factors in Microtubule Nucleation

Jake Adkins
Advisor: Sabine Petry

During cell division, microtubules are essential to form a large dynamic array called the mitotic spindle which functions to physically segregate the chromosomes. To accomplish these functions, the nucleation and dynamics of microtubules must be carefully regulated in both space and time. Historically, gamma-tubulin has been widely thought of as the universal MT nucleator of the cell. However, gamma-tubulin activity alone cannot fully explain the nucleation potential of the cell, suggesting the presence of unknown components in the nucleation mechanism. This thesis focuses on the structure of two proteins – XMAP215 and TPX2 – which have both been recently demonstrated by our lab to mediate the nucleation of microtubules, primarily through the activity of their C-termini. The structure of the C-terminus of XMAP215 and the site of its interaction with gamma-tubulin is investigated. In these investigations, the TOG5 domain within the C-terminus of XMAP215 is demonstrated to have a key role in the stability of the protein. The structure of the C-terminus of protein TPX2 is also investigated through a series of crystallization trials. Through these trials the ability of the TPX2 C-terminus to drive phase separation is displayed, and guidelines are proposed to circumvent the difficulties posed by phase separation in future experiments. A crystal hit was also obtained during crystallization trials, the identity of which is investigated in X-ray diffraction studies.

Investigation of Determinants Governing Hepatitis E Virus (HEV) Host Tropism and Identification and Characterization of Small Molecules Inhibiting HEV Replication

Nicholas Archer
Advisor: Alexander Ploss

Each year, hepatitis E virus (HEV) infections lead to an estimated 14 million symptomatic cases, resulting in approximately 300,000 human deaths and 5,200 stillbirths. While mortality rates in healthy individuals are around 2%, HEV causes death in ~30% of infected pregnant women, as well as chronic hepatitis in the immunocompromised (e.g. organ transplant recipients and HIV-positive patients). HEV is transmitted faeco-orally, often through contaminated water, and zoonotically, via consumption of undercooked pork meat. These modes of disease spread, along with nosocomial transmission from organ and blood donations, contribute to the status of HEV as a global threat to public health. The few explored medical treatments against HEV infection cause severe side effects while remaining only partially effective and unsafe for pregnant women and fetuses. My thesis addresses two important and unmet needs in the field: firstly, given the lack of a susceptible small animal model for in vivo HEV experimentation, we aimed to identify the determinants of HEV host tropism, particularly the basis for murine resistance. To achieve this, we developed a human-murine heterokaryon assay to discern whether positive human host factors or negative murine restriction factors are responsible for the observed tropic limitations of HEV. Our data suggest that one or more endogenous negative restriction factors in murine cells account for the inability of HEV to complete its life cycle in a murine host. Identification and inactivation of such (a) putative, antagonizing element(s) may aid in the development of an urgently needed mouse model suitable for antiviral drug testing and mechanistic studies of HEV-induced immune responses and pathology. Secondly, we aimed to identify compounds with novel antiviral properties against HEV infection. We designed and executed a high-throughput screen to select potential therapeutic candidates from a library of small molecules. In doing so, we identified a single promising hit that is non-toxic at functional doses and more potent than existing drug options. Our current efforts involve determining the drug’s mechanism of action, exploring the efficacy of closely related structural analogues, and testing the drug in physiologically relevant cell culture and animal models.
Endogenous Visualization and Manipulation of Genes in the Gap Gene Regulatory Network in Developing D. melanogaster

Ruchita Balasubramanian
Advisor: Thomas Gregor

The gap gene network is critical for anterior-posterior patterning of the developing Drosophila melanogaster embryo. Gap genes are well characterized and provide an excellent platform to investigate endogenous transcription in real-time. While the synthetic reporter systems are valuable for real-time visualization of gap gene transcriptional activity, they fail to capture endogenous transcription, obscuring context dependent factors that affect transcription, including potential network effects. Here, we utilize a novel CRISPR based methodology that allows us to both introduce a tagging system to visualize endogenous gap gene transcription in real-time, and also manipulate regulatory regions of genes in the network. To gain insight into the effects of multiple enhancers on endogenous gene expression, we utilize this methodology to visualize the phenotypic effects of deletions of the proximal and distal enhancers that govern kni expression on early embryonic development and adult viability. We find that the distal enhancer appears to be more strongly implicated in establishing the posterior domain of kni expression and wildtype development than previously thought. Most importantly, we find that the proximal and distal enhancers do not behave as discrete, modular units but rather interact synergistically to establish proper kni expression. We also addressed whether differences in expression of X-linked genes are occurring during early development by comparing endogenous transcriptional activity of gt in male and female embryos. We observe differential expression between sexes occurring in the posterior domain of gt expression between nuclear cycles 13 to 14. Finally, we conclude with a first demonstration of the visualization of simultaneous endogenous transcriptional activity of three gap genes: Kr, kni and gt. Together our results open new avenues for future study of the dynamics of regulatory interactions between multiple genes in a wild-type background. We broadly demonstrate the capability of our methodology to investigate aspects of endogenous transcription that can be widely applied to other metazoan systems.

repx-I Regulates Reproductive Aging in Caenorhabditis elegans

Julia Casazza
Advisor: Coleen T. Murphy

As more women choose to start families at later ages, age-associated infertility becomes increasingly relevant. While little is known about the genes that control reproductive aging, the model organism Caenorhabditis elegans provides an excellent platform to study this problem. Previous work in The Murphy Lab identified the receptor tyrosine kinase repx-1 as a regulator of reproductive span. This thesis strives to understand the mechanism through which loss of repx-1 extends reproductive span. We report that repx-1 loss extends reproductive span via oocyte quality maintenance. repx-1 does not extend lifespan but does alter developmental timing. Transcriptional changes in repx-1 mutants closely resemble those of crh-1 (CREB homolog) mutants, which also experience extended reproductive span but not lifespan. Many of the genes overexpressed in repx-1 mutants are implicated in fat metabolism, including a subset that is enriched for the HLH-30 (Helix-Loop-Helix 30, the C. elegans ortholog of mammalian Transcription Factor EB) binding motif. Loss or knockdown of hlh-30 further extends reproductive span in reproductive longevity mutants repx-1 and daf-2. Our results characterize repx-1 as a regulator of reproductive longevity and implicate hlh-30 in reproduction. Future work should decipher the genetic relationship between repx-1 and crh-1 through epistasis analysis. Additionally, future work should investigate how hlh-30 knockdown extends reproductive span via changes to lipid metabolism.
P-element Induced Wimpy Testis (PIWI) Expression in the Central Nervous System of Ciona intestinalis is Moderated by Transcription Factors Otx and Bsh

Annie Choi
Advisor: Michael Levine

Ciona intestinalis is an ascidian species that has been a popular subject of research due to its proximity to vertebrates in evolution. The motile, swimming larvae of Ciona turn into sessile adults via metamorphosis, requiring the regeneration and restructure of cells after reaching maturity. Recent research suggested that ependymal cells in the Ciona central nervous system (CNS) may serve as neural stem cells by demonstrating that part of the adult neurons differentiate from these cells. Our lab’s single-cell RNA transcriptomics data has shown P-element–induced wimpy testis (PIWI) expression in the CNS, including some overlapping expression with the ependymal marker opsin3. Based on previous reports of PIWI function in maintaining genome integrity in the germline and neural progenitor cells, I investigated the regulation of PIWI expression in the Ciona central nervous system. Serial deletion of the endogenous piwi 5 kb intronic region led to a 300 bp region that drove expression. I have analyzed binding motifs in the 300 bp region of piwi enhancer and found multiple Otx binding sites. Otx overexpression and target gene repression, as well as binding site mutations, suggested that Otx directly binds to the piwi enhancer to activate transcription. I have also discovered that brain-specific transcription factor Bsh acts upstream of piwi to repress expression. Surprisingly, evidence shows that Otx activates bsh as well as piwi, suggesting a regulatory mechanism that modulates piwi expression. By investigating how piwi is regulated in the CNS, this study opens up the question of whether the properties of progenitor-like ependymal cells can be explained through known piwi function.

Dietary Supplementation of Oleic Acid Rescues Mating-Induced Death in Caenorhabditis Mothers

Leo Choi
Advisor: Coleen T. Murphy

Increase in reproduction via mating is associated with decrease in lifespan in many organisms. Yet, methods to mitigate this mating-induced lifespan reduction have not been investigated. Here, we dietarily supplemented Caenorhabditis elegans with fatty acids as a potential method to restore lifespan of mated mothers. We discovered that supplementation of oleic acid fully rescues mating-induced death and fat loss. Oleic acid supplementation did not affect the reproductive span and the progeny production of mated worms. We further found that supplementation of other fatty acids does not rescue lifespan, and that endogenous oleic acid protects worms against mating-induced death. Such lifespan rescue through oleic acid supplementation is conserved, in C. elegans hermaphrodites that lack self-sperm and in gonochoristic C. remanei females. Our study provides a novel mechanism of lifespan rescue and reveals a unique role of oleic acid as regulator of somatic health in mated animals.
Regulation of the VqmA-VqmR quorum-sensing pathway in *Vibrio cholerae*

Daniela Coronado
Advisor: Bonnie Bassler

The global crisis of antibiotic-resistant bacteria demands the search for new alternatives to traditional antibiotics. Bacteria like *Vibrio cholerae* can form communities in self-produced matrices, called biofilms, that facilitate the sharing and sensing of chemical signals and public goods. Some of the chemical signals are autoinducers that bacteria produce, detect, and collectively respond to in the process called quorum sensing (QS). Through QS, bacteria coordinate the expression of virulence factors and biofilm formation genes. Thus, understanding how *V. cholerae* regulates its QS-controlled biofilm lifestyle could form the basis for the development of new antibacterial treatments. In *V. cholerae*, the QS autoinducer 3,5-dimethylpyrazin-2-ol (DPO) binds to and activates the receptor VqmA, which as a complex, activates vqmR expression. VqmR is a small RNA that represses genes required for biofilm formation. Deletion of the threonine dehydrogenase (tdh) gene encoding an enzyme involved in the synthesis of DPO does not fully inhibit vqmR expression, while deletion of the vqmA gene does. This finding suggested that there exist mechanisms of vqmR regulation that are independent of DPO. Mutagenesis and biochemical analyses demonstrate that the absence of phosphotransacetylase (Pta) and the cAMP-receptor protein (CRP) increase expression of vqmR in strains that do not produce DPO. The effect of CRP on vqmR occurs via reduction of VqmA protein levels, while the Pta activity is likely via post-transcriptional regulation of VqmA, which can be rescued by supplementation with acetate or acetyl phosphate. Together, these data provide a connection between the sensing of carbon and acetate availability and QS.

Does Animal Development Follow the Arrhenius Equation?

Joseph Crapse
Advisor: Martin Wuhr and Eric Wieschaus

Since the late 19th century we have described the dependence of simple chemical reaction rates (*k*) on temperature (*T*) with the Arrhenius equation: $k = A \exp(E_a/k_bT)$, where $A$ is the frequency factor, $k_b$ the Boltzmann constant, and $E_a$ the activation energy. Recent evidence suggests that more complicated reaction networks in biology, e.g. simple biological processes also follow this simple relationship. Here I carefully investigate the temperature dependence of animal development in frog model embryos. To this end, I recorded development of the frog *X. laevis* from the fertilized egg to the onset of muscle movement at various temperatures. My data shows that frog development is well described by the Arrhenius equation in a temperature range from 16-23.5 degrees Celsius, regardless of developmental stage analyzed. One simple explanation for this may be that evolution led all rate-limiting activation energies in frog to converge. However, my data shows that different stages of development exhibit significantly different apparent activation energies. Despite these different activation energies, the summed sequence of development continues to follow the predictions of the Arrhenius equation. This finding seems to contradict the simple underlying individual molecular assumption of the Arrhenius Equation, which starkly contrasts with the complexity of embryonic development. To address this question, I simulated coupled reaction networks wherein the individual steps follow the Arrhenius equation and observed how the entire network rate scaled with temperature. I show analytically, that an entire reaction network cannot be described perfectly with the Arrhenius equation when individual activation energies differ. However, for relevant biological temperature ranges and activation energies, the cumulative behavior of the network can be approximated by the Arrhenius equation, likely well within the achievable error bars of biological experiments. Thus, I was able to observe that the dependence of embryonic development rate on temperature is well described with the Arrhenius equation. Furthermore, I present an analytical framework that demonstrates how highly complex biological networks follow this simple rule.
Non-communicable diseases are the leading cause of early death and disability worldwide. Among these diseases, idiopathic scoliosis affects approximately 4% of the world’s seemingly healthy children. Additionally, the burden of patient care is significant; hospital stays for a child affected with scoliosis cost five times more than the average hospital stay. Despite these considerations, we have seen a decrease in school screening for the disease and a lack of biological and epidemiological research regarding the condition. Consequently, we know very little about the etiology of the disease, let alone a possible cure. Animal models can help advance our understanding of disease, and the Burdine lab previously used zebrafish to demonstrate an association between cilia motility and spinal curvature in a model of idiopathic scoliosis. I took advantage of the external development, transparency and genetic tractability of zebrafish to probe the heterogeneity of cells expressing motile cilia at juvenile stages. Single cell RNA sequencing of cells with motile cilia, followed by clustering analysis, revealed a large population of radial glial and neural progenitor cells. Notably, there was an absence of cells expressing ependymal markers. This suggests that either a technical issue prevented our identification of this cell type, or that ependymal cells are not the prime motile ciliated cells lining the brain ventricles and spinal canal at this stage. The second goal of my project was to generate zebrafish mutant for the idiopathic scoliosis candidate gene POC5, which has common single nucleotide variations among affected individuals, using CRISPR/Cas9 strategies. We were able to generate a POC5 mutant zebrafish with a truncation in the protein that led to scoliotic-like curvature. My studies therefore advance our understanding regarding the motile ciliated cellular composition of the central nervous system. Furthermore, we generate more zebrafish with defective cilia to further elucidate the role of cilia in maintain spine linearity. Overall, I have laid the groundwork for continuing studies on the etiology of idiopathic scoliosis, which is necessary for the ultimate transfer of research achievements to therapeutic treatment development and clinical practices.

Spondylometaphyseal dysplasia (SMD) is a group of skeletal disorders characterized by deformities of the spine and long bones. While the etiology of many SMDs are unknown, the discovery of a novel fibronectin mutation (FNC97W) has been recently linked to an individual with SMD. Fibronectin (FN) is a vital extracellular matrix (ECM) protein that assembles into a fibrillar matrix and regulates cellular activities, including matrix assembly of other ECM proteins such as type I collagen (COL1). Proper assembly of the ECM is essential for normal cell and tissue development, providing organization, developmental signals, and structural support to cells. Mutations in COL1 and other ECM proteins have been linked to skeletal disease through a number of different mechanisms, including deregulated matrix assembly and perturbations in cellular function caused by protein misfolding and subsequent ER stress, suggesting pathways by which our novel FN mutation may lead to SMD. Analyses of primary dermal fibroblasts heterozygous for the FNC97W mutation reveal abnormal accumulation of FN within the ER of the cells, which suggests ER stress and activation of the unfolded protein response (UPR), as well as lower levels of FN in the culture medium, revealing a defect in FN secretion. The cells also assemble a reduced FN matrix and fail to assemble a COL1 matrix. These results provide new insights into the effects of a FN mutation on cellular function, highlighting the importance of FN and COL1 matrix assembly in skeletal development and elucidating their potential role in the pathogenesis of SMD.
The Non-Canonical Role of TERT: A Potential Mechanism for Progesterone-Mediated Neuroprotection After Moderate Traumatic Brain Injury

Kerry Farlie
Advisor: Daniel Notterman

Traumatic brain injury (TBI) is a prevalent cause of death and disability in the United States, affecting an estimated 1.7 million individuals each year. Due to the complexity of the mechanisms underlying brain injury, there is currently no effective, specific treatment for moderating the underlying molecular events that link an injury to the ultimate functional outcome. Recent literature highlights progesterone as a candidate treatment for TBI due to its neurogenerative and neuroprotective properties, but the mechanism by which progesterone imparts neuroprotection remains elusive. Progesterone is thought to regulate telomerase activity (TA), and based on preliminary experiments, we hypothesized that there is an optimal dose of progesterone that maximally increases TA. Canonically, telomerase functions to elongate telomeres, the sequences at the ends of chromosomes that protect genetic information during cell division. However, TERT, the catalytic subunit of telomerase, has also been found to participate in non-canonical roles, including protecting cells from oxidative stress and promoting neuronal survival. We analyzed the effects of different doses of progesterone in naïve, sham injured, and cortically-impacted mice 1 day and 14 days after cortical contusion injury, which mimicked a moderate TBI. Here we report upregulation of TA after treatment with 8 mg/kg of progesterone and suppression of TA after treatment with 32 mg/kg in three distinct brain regions, consistent with the hypothesis that supraoptimal dosages of progesterone could be harmful to neuro-recovery. We also measured telomere length (TL) because shorter TL has been associated with oxidative stress, a result of TBI. TA and TL did not appear to be correlated, suggesting that TBI might trigger the non-canonical neuroprotective activity of telomerase. Our findings support a novel mechanism by which progesterone confers neuroprotection by modulating the activity of TERT, specifically through its non-canonical functions, to attenuate stigmata of TBI.

Exorcising the Noonday Demon: Esketamine and Other Novel Approaches for Addressing Treatment-Resistant Depression

Jessica Goehring
Advisor: Daniel Notterman

Esketamine is a newly approved glutamatergic antidepressant that acts through a novel pathway not addressed by most drugs adhering to the monoamine hypothesis. This study aims to utilize the literature to connect the many different theories about the mechanism of the pathophysiology of depression into a single chart that marks where common antidepressants begin effecting change in the pathways in question. This will serve as a point of comparison between entry points of monoaminergic antidepressants and glutamatergic antidepressants. There is also potential for different interpretations of the mechanism of action of ketamine because while esketamine was the enantiomer approved by the FDA, there is evidence supporting a metabolite of the R-enantiomer (2R,6R-hydroxynorketamine) as the epitome of therapeutic efficacy for ketamine through its interaction with the AMPA receptor. By evaluating the mechanistic implications of the literature, we can then propose recommendations for drug discoverers to develop faster-acting antidepressants. Ideally, antidepressants that are as effective and fast-acting as esketamine won’t just be used for TRD, but will be a first line treatment once the side effects and recreational abuse potential of future drugs like esketamine are reduced.
Designing a System to Investigate the Impact of Spatial Transcriptional Differences in the Liver on Hepatitis Delta Virus Infection
Raymond Guo
Advisor: Alexander Ploss

Hepatitis B virus (HBV) and the satellite hepatitis delta virus (HDV) cause liver infections which affect millions worldwide. HBV/HDV co-infection of human liver hepatocytes, which are important to metabolism, causes accelerated liver damage and elevated risk of hepatocellular carcinoma. Environmental cues affect hepatocytes’ transcriptional profile, and transcriptional differences divide the liver into zones. However, it is unclear if all hepatocytes are equally susceptible to HDV infection, and we hypothesize that liver zonation patterns distinctively affect permissiveness to viral infection. The Ploss Lab has generated a novel genetically humanized mouse model supporting HDV infection, and in order to utilize this mouse model to investigate the effects of liver zonation on HDV infection, we have designed a novel reporter system fusing the only gene product of HDV, the hepatitis delta antigen (HDAg), to Cre recombinase to enable permanent genetic marking of hepatocytes that take up this “HDVLP-Cre.” HDV-Cre DNA in vitro transduction experiments and hydrodynamic delivery of DNA plasmid to humanized mice confirm recombinase activity, and in vitro HDVLP-Cre infections of reporter cell lines demonstrate overall reporter activation. However, packaging of HDVLP-Cre proved to be difficult yielding relatively low reporter activity of the HDVLP-Cre construct which hampered more significant progress. Efforts to improve overall reporter activity included accelerating the experimental time frame as well as investigating alternative reporter cell lines, which yielded varying amounts of increased reporter activation. Moving forward, the production of HDVLP-Cre and overall HDV-Cre reporter construct will be further optimized for use in humanized mouse infections. Following in vivo infections of humanized mice, a potential unique zone of hepatocytes susceptible to initial HDV infection may be identified by analyzing hepatocytes which are permissive to HDVLP-Cre infection, allowing us to further investigate host factors which promote the establishment of HDV infection. The implications of this project can thus lead to future studies of targeted therapeutic treatment of HBV and HDV.

Overstepping Boundaries: Hotspot Mutation in GATA3 Promotes EMT and Metastasis in Breast Cancer
Ruby Guo
Advisor: Yibin Kang

GATA3, a critical transcription factor in mammary gland development, has traditionally been regarded as a tumor suppressor in breast cancer. Mutations in GATA3, which are commonly believed to function through the elimination of these tumor suppressive effects, occur in over 10% of all breast cancer patients. This study aims to characterize a previously unexplored hotspot mutation that contributes to one-fifth of all GATA3 mutations. Strikingly, rather than a loss of function, we find that this mutation produces a truncated, mutant GATA3 protein that confers an oncogenic gain of function. In cells, we observe that mutant GATA3 promotes migration and invasion, as well as epithelial-mesenchymal transition (EMT), which are characteristics that have been linked to increased metastatic potential in tumor cells. Consistent with these findings, we show that the mutant GATA3 promotes lung metastasis in several mouse models. A closer look at the primary tumors in these mice reveals a disorganization of tumor boundaries, presumably wrought by the invasion of tumor cells into surrounding tissues. We also gain valuable insight into mutant GATA3’s mechanisms by identifying its protein interactions, as well as direct downstream gene targets. Taken together, these findings suggest that mutant GATA3 may serve as an early prognostic marker for breast cancer and provide a potential therapeutic target. These tools are relevant towards addressing the rising breast cancer epidemic in a number of countries, which we discuss in the context of the urban-rural fabric of India. This work is an exciting step forward towards promoting the health and dignity of breast cancer patients globally.
Improving the Drug Development Process: A New Paradigm for Preclinical Development
Laura Halsey
Advisor: Jane Flint

In this thesis, the current process of pharmaceutical development in the United States is shown to require significant investment in terms of cost and time, which by extension causes high drug prices and reduced innovation in the pharmaceutical industry. The implications for drug accessibility and unmet medical need as a result of prohibitively high drug prices and low pharmaceutical innovation warrant attention and scholarship on this topic. I analyze the process of drug development for possible inefficiencies contributing to its excessive cost and timeline, and create a multi-faceted recommendation to reduce inefficiency by applying recent advances in molecular biology; this could then lower drug prices and increase pharmaceutical innovation. Preclinical development, which occurs after drug candidate discovery and before clinical trials, is determined to be the most effective stage upon which to focus improvement efforts because of significant resource saving potential and inherent flexibility in this stage. If, through improved preclinical testing, earlier attrition of unsafe or ineffective pharmaceuticals occurs and promising drugs are better optimized for human use in clinical trials, cost and time investments would be reduced. I propose three categories for improvement in preclinical testing: data-sharing and FDA communication shortcomings, biological and physiological knowledge base gaps, and technology deficits. Recommendations and paradigm shifts are designed for each category with the aim to make preclinical development, and by extension the entire drug development process, more efficient.

Genetic and Environmental Determiners of Longevity
Isabel Hsu
Advisor: Jane Flint

Humans have tried to prolong life since ancient times, and life spans have been steadily increasing throughout history. Longevity is heritable: Relatives of exceptionally long-lived people are more likely to live longer lives. Thus, it is hypothesized that there are certain genes associated with longevity. Through a search of model organism gene databases, four human gene homologs were identified as longevity genes in mouse, fly, worm, and yeast. These are thioredoxin, superoxide dismutase 1, peroxiredoxin 1, and sirtuin 1. In addition, genes that exist in human and are associated with longevity in model organisms were examined. Many of these longevity genes are involved in protection against oxidative stress, cancer prevention, and the insulin/insulin-like growth factor signaling (IIS) pathway. Lastly, the environment also influences longevity. Factors in early life and adulthood are considered, and changes in the epigenome and microbiome are discussed.
A Comparative Analysis of HSV-1 and PRV Transcriptional Activator VP16 in Primary Neurons
Zhi-Shan Hsu
Advisor: Lynn W. Enquist

The VP16 tegument protein of herpes simplex virus 1 (HSV-1) has been shown to have a role in reactivation of latent infection in the peripheral nervous system (PNS), but while it appears to activate viral gene transcription, it is unknown if this protein can also activate neuronal genes. Less research has been done on the VP16 homolog in the related pseudorabies virus (PRV) and any role it may play in activating neuronal genes. By using adeno-associated virus (AAV) vectors that encode either HSV VP16 or PRV VP16 (aka UL48), cultured superior cervical ganglia rat neurons (SCGs) can be transduced and made to express VP16 or UL48 independent of virus infection. Gene expression in SCGs transduced in this manner was compared using RNA-seq and RT-qPCR and it was found that the neuronal gene Jun was enriched in the presence of HSV VP16, Adcyap1 with PRV UL48, and Crem in the presence of both proteins. Subsequent analysis of subcellular localization in AAV vector-transduced and virus-infected SCGs showed that, while localization of Adcyap1 and Jun did not change with or without the presence of the VP16 proteins, Crem was nuclear only in the presence of PRV UL48. It appears that PRV UL48 may be increasing expression of Crem and Adcyap1 but only recruiting Crem to the nucleus for activation of viral gene expression. While the presence of HSV VP16 is connected to enrichment of Crem, that same nuclear localization is not observed, suggesting it may not play the same role in HSV-1 as in PRV.

Tuning In: How Erk Dynamics Influence Downstream Gene Expression in Mouse Keratinocytes
Danielle Isakov
Advisor: Jared Toettcher

The Ras/Erk mitogen-activated protein kinase (MAPK) pathway is a central signaling pathway that is involved in diverse cell fates such as proliferation, differentiation, and apoptosis. Although the molecular players of the pathway have been extensively characterized, the method by which the cell uses a single pathway to interpret varying inputs into different downstream outputs is still unknown. Uncovering this missing process of regulation is crucial for understanding not only natural phenomena, but also pathogenic cellular behavior, as mutations in the Ras/Erk pathway account for nearly a third of all cancers and 80% of melanomas. Our lab is interested in one dimension of control—dynamics of Erk activity. Changes in Erk oscillations in cells have shown to result in different downstream cellular outcomes, but whether and how these oscillations inform transcription of downstream gene targets remains an open question. Primary mouse keratinocyte epithelial cells display endogenous pulsatile Erk activity and exhibit strong proliferation and differentiation behaviors, making them an ideal candidate for studying the significance of Erk dynamics. In my thesis work, I built a system for live-cell reporting of pulsatile Erk activity and transcriptional activity of the immediate-early gene Fos in mouse keratinocytes. My findings show that Erk oscillations, whether intrinsic or environmentally induced, lead to transcriptional activity of the Fos promoter. Moreover, drugs that target the Ras/Erk pathway induce changes in pulsatile Erk activity that is carried through in transcriptional changes. My findings expand on the role of Erk dynamics in determining downstream cell-fates, and my project provides promising future directions for continued exploration of signaling dynamics in keratinocytes.
Epigenetic and Phenotypic Effects of Fracking Exposure in Children from the Fragile Families and Child Wellbeing Study

Benjamin Jacobson
Advisor: Daniel Notterman

The development of hydraulic fracturing (fracking) for natural gas over the past decade has radically transformed the nature of U.S. energy, playing a large role in U.S. energy independence. However, these accomplishments have led to a large increase in fracking site development with little regulatory oversight or exploration of consequences of this technique on the health of nearby residents. While recent studies have begun to suggest significant health risks correlated with fracking exposure, the fact that fracking has only emerged in the past few years has limited the number of extensive, longitudinal studies undertaken. Data for examining the epigenetic impacts of fracking exposure are only now becoming available. This study analyzes DNA methylation and phenotype data collected just before the fracking surge and again after six years of sustained fracking development, in an effort to help fill the current gap in understanding. I find suggestive evidence of increasing BMI and accelerating methylation age in children exposed to fracking between the ages of nine and fifteen. I further identify CpGs associated with four genes, ZNF644, RAD18, NAT1, and ABCA1, which appear differentially methylated after fracking exposure. Finally, I identify a gene, DPYD, which contains five differentially methylated CpGs, as well as other genes with multiple differently methylated CpGs that are implicated in weight gain and toxicant efflux. These findings should encourage further research into the epigenetic effects of fracking. This paper also presents a novel measure of fracking exposure and an analytical framework that will be useful in subsequent work that explores potential links between fracking exposure and DNA methylation.

Structural Characterization of the Sterol-Sensing Domain in the NPC1 and SCAP Membrane Proteins

Joyce Lee
Advisor: Nieng Yan

Sterols are essential biomolecules in animals that aid in hormone and bile acid synthesis, and serve as key structural components of the cell membrane. Cholesterol homeostasis heavily regulates cellular levels of sterols, but this biochemical process remains unclear. It is thought to be controlled by several membrane proteins, including Niemann-Pick disease type C-1 (NPC1) protein and sterol regulatory element-binding protein (SREBP)-cleavage-activating protein (SCAP). These proteins’ functions are dependent on a highly-conserved sterol-sensing domain (SSD) that is thought to regulate interactions with sterols. However, there is no direct evidence of the mechanisms of the SSD in the aforementioned proteins. To overcome the inherent challenges of studying membrane proteins, cholesterol, and their exact interactions, single-particle electron microscopy (cryo-EM) was used to provide structural insights on both cholesterol homeostasis and other processes involving SSDs. The goal of the investigation is to capture high-resolution images of NPC1’s SSD binding interface with cholesterol, as well as the sterol-dependent interactions between Insulin induced gene-1 (INSIG1) and SCAP, which also occurs through the SSD. We present several constructs of human SCAP that indicate detectable protein expression levels and stability. Furthermore, a 6.9 angstrom (Å) structure of NPC1 in nanodisc has been resolved with no sterol density indicated in the predicted binding pocket of the SSD. These results provide deeper insights on the complexity and dynamics of proteins involved in cholesterol metabolism and signaling. Optimization of the construct designs and cryo-samples will be necessary in future experiments to ultimately image these proteins and their interactions at the SSD.
Investigating the Activation and Asymmetric Regulation of \textit{dand5} in Zebrafish

Sally Lee
Advisor: Rebecca Burdine

Establishing the left-right (L/R) axis in the vertebrate body is a crucial step in proper development, as it governs the asymmetric positioning and formation of the organs and vasculature. The L/R axis is established in the early embryonic stages through the breaking of symmetry at the left right organizer. In the zebrafish organizer, Kupffer’s vesicle (KV), cilia generate directional fluid flow that results in the preferential right-sided expression of \textit{dand5}.

\textit{dand5} is a key repressor of the Nodal signaling cascade, which leads to formation of left-side morphology. However, the factors that are responsible for asymmetric \textit{dand5} expression remain elusive. Previous studies have demonstrated that the 1.2kb upstream regulatory region (referred to as the URR) and the 3’ untranslated region (UTR) are essential for driving \textit{dand5} transcription and asymmetric expression, respectively. Building on these findings, this work first aims to identify the regulatory sequences that are necessary and sufficient for \textit{dand5} transcription, using a series of Tol2 transposon-mediated URR deletion assays in the zebrafish embryo. Injections of plasmids containing URR-driven EGFP demonstrate that we have a working model for visualizing \textit{dand5} transcriptional activation. Second, this study investigates the cis-regulatory elements responsible for the post-transcriptional asymmetric regulation of \textit{dand5} expression, using CRISPR/Cas9 to generate mutants with deletions along the 3’UTR. Following deletions of specific regions within the URR and 3’UTR, \textit{dand5} expression was analyzed via immunofluorescence and in situ hybridization. The findings of this study expand our current understanding of asymmetric determination and organogenesis, and provide new insights into the molecular mechanisms that underlie major laterality defects, such as congenital heart diseases.

Investigating the Interaction Between the Sec1/Munc18 (SM) Protein Vps33 and its Qa-SNARE Vam3 and R-SNARE Nyv1

Sarah Lee
Advisor: Frederick Hughson

SNAREs zipper together to form four-helix membrane-bridging complexes that promote membrane fusion. SNARE complex formation is regulated by Sec1/Munc18 (SM) proteins. SM proteins interact with SNAREs, but exactly how they regulate SNARE complex assembly is not well understood. Previously, our lab reported separate crystal structures of the SM protein Vps33 bound to its R-SNARE Nyv1 and to its Qa-SNARE Vam3. Superposition of the two structures suggested that Vps33, and potentially other SM proteins, could serve as templates to bring the R- and Qa-SNAREs together to form a half-zippered SNARE complex. However, no structure of an SM protein bound simultaneously to its Qa- and R-SNAREs has been reported for any SM protein. A structure of the ternary complex will reveal whether and, if so, how Nyv1 (R-SNARE) and Vam3 (Qa-SNARE) influence each other’s binding to Vps33 as they initiate SNARE complex assembly. In this thesis, we explored a novel strategy of purifying Nyv1 and Vam3 as disulfide-linked heterodimers to obtain a crystal structure of the ternary complex. This strategy has been previously shown, through single-molecule force spectroscopy, to be compatible with template complex formation. Using this approach, we identified three conditions that showed crystal growth. Diffraction data were collected from optimized crystals of one of the conditions. Biochemical analyses were performed to better characterize the interaction between Vps33 and its SNAREs. Addition of Vps33 enhanced the formation of covalent Nyv1/Vam3 heterodimers. This provides new evidence in support of the model that Vps33 serves as a template for the assembly of Nyv1 and Vam3. In addition, isothermal titration calorimetry assays were performed to estimate the dissociation constants between Vps33 and Nyv1 and Vps33 and Vam3. Taken together, our findings suggest that engineered disulfide bonds may represent a promising strategy for structurally characterizing SNARE assembly pathways.
**Lost In Translation: Investigating Translational Repression With Optogenetic RNA Clustering**

Lian Kirit Limperis

Advisor: Jared Toettcher

RNA translation is a highly regulated and integral process for all living cells. In recent years, researchers have been investigating the role of phase separated membraneless organelles – where RNA and proteins can co-localized – in translational control. These RNA-protein bodies (RNPs) in and of themselves are a diverse system that plays roles in single cells as well as in developing organisms. While many studies have been conducted on RNPs in vitro, little research has been performed to investigate their linkage with translational regulation in live cells. Here we applied optogenetics to create a controllable RNA-protein clustering system in NIH 3T3 cells to see whether RNP phase separation can regulate translation. We then quantified the translation of a fluorescent reporter in the presence and absence of clustering. The building of this controllable RNA-protein clustering system allows for further investigations into the role of phase separation in RNA translational regulation and development.

**Towards *in vitro* and *in vivo* Model Systems for Hepatitis B and Delta Viruses**

Gabriel Lipkowitz

Advisor: Alexander Ploss

Hepatitis B virus (HBV) is one of the oldest viruses known to afflict humankind, having been discovered in the remains of ancient Egyptian and Korean mummies. Today, HBV remains a global scourge, infecting approximately 257 million individuals worldwide, of whom about 887,000 die of complications each year. Besides fundamental interest into the basic biology of this virus, a need to develop curative therapeutics motivates biomedical research. For the past several decades, study of this virus and development of therapies has been hampered by a severe lack of model systems, both in vitro and in vivo. While in vitro systems cannot support robust infection for more than a week, there exist few in vivo systems for the virus at all, save for the chimpanzee, research on which a recent NIH moratorium halted, and the transgenic mouse, which while certainly helpful is genetically dissimilar to humans. In this thesis, we make progress on both the *in vitro* and *in vivo* fronts. For an *in vitro* model, we employ the self-assembling primary human hepatocyte co-culture (SACC-PHH) model system, which takes advantage of non-parenchymal fibroblasts to allow primary human hepatocytes to support HBV infection for up to four weeks, far longer than current systems and without suppression of antiviral immunity. We utilize this model for several applications, including co-infection with the satellite hepatitis Delta virus, testing of antiviral therapeutics, RNA sequencing, and mathematical modeling of infection. For an *in vivo* model, we pursue a viral adaptation project. Here, we mutagenize residues of the HBV surface protein, preS1, to discover variants capable of utilizing a cynomolgus macaque hepatocyte receptor for entry. Altogether, we anticipate that our results will empower other researchers to pursue some of the most pressing questions in HBV biology today, whether they concern the host immune response to infection, transcriptomic changes undergone by infected hepatocytes, or the development of curative therapeutics.
The Role of Purkinje Cells in Cognitive and Affective Development

Christina Matl
Advisor: Samuel Wang

Discovery of reciprocal cerebello-thalamo-cortical loops have substantiated a link between specific lobules within the cerebellum and the cognitive and affective functions associated distant forebrain regions. Additionally, abnormalities in these loops are thought to be involved in the pathogenesis of Autism Spectrum Disorder (ASD). This study investigates cerebellar mechanisms by which experience guides cognitive and affective functions in distant brain regions. Purkinje cell activity was manipulated in either lobule VI or crus I to determine what role Purkinje cell signaling plays in the maturation and exhibition of social preference and flexible learning. Purkinje cell activity was perturbed acutely in adulthood to probe lobule-specific roles of Purkinje cells during behavior. Purkinje cell activity was perturbed chronically during development to assess how Purkinje cells influence neocortical development. Wireless optoelectronic devices were used to stimulate lobule VI Purkinje cells in a precise temporal manner during social chamber testing to probe whether decreasing cerebellar output in a temporal fashion alters social behavior. Acute and developmental perturbation was achieved using Designer Receptors Exclusively Activated by Designer Drugs (DREADDs). Acute DREADD inhibition of Purkinje cells in both lobule VI and crus I lead to a profound decrease in flexible learning. Furthermore, Acute DREADD expression in lobule VI showed a slight increase in social preference. Optogenetic stimulation of Purkinje cells in lobule VI during moments of mouse-novel mouse or mouse-novel object interaction lead to pronounced indifference between the novel mouse and novel object.

Imperfect Match: Modeling Physiological Stress in Individuals of Discordant Self-Reported Race and Genetic Ancestry

Stephanie Ohagi
Advisor: Dalton Conley

In the United States, racial classification of African Americans is derived from the ‘one-drop rule’ and currently practiced through phenotypic presentation, but African Americans, having undergone recent genetic admixture, do not consist of only one geographic ancestry group. While previous research has also concluded that individuals may self-identify as one race but contain contradictory genetic ancestry, there is no literature that discusses whether or not living one’s life with this inconsistency may be associated with an increased genetic predisposition to stress-related health consequences, such as high blood pressure and diabetes. In this study, I sought to determine if ancestrally-deviant African Americans and White Americans had a higher risk and stronger predictive power for physiological determinants of chronic stress compared to their ancestrally-concordant counterparts. To do so, I constructed a cohort study consisting of ancestrally-deviant and concordant African Americans and Caucasians (n = 1,076, 1,122, 6,444, and 8,920 participants, respectively) using genomic, demographic, and physiological data from the Health and Retirement Study (HRS). I then chose nine dependent variables based on current research that links them to long-term stress. Cohort creation via genetic ancestry principal component thresholds, model construction, statistical analysis, and subsequent data presentation were all performed in R. There was a significant robustness in the type 2 diabetes (T2D) and BMI models in both ancestrally-deviant and concordant African American cohorts, seen in their corresponding adjusted R2 values (T2D; R2 = 0.575, 0.230 | BMI; R2 =0.237, 0.117). The models also predicted a positive correlation between AFR ancestry and BMI and T2D, suggesting that these two particular dependent variables may be more integral to determining the effects of discordant genetic ancestry and self-identified race and ethnicity (SIRE). While a majority of the models proved to be either inconclusive or have low predicting power, the contribution of this paper to both the literature
concerning geographic ancestry as well as the current pharmacogenetics industry positions itself as a relevant
guideline for future related works.

**How do Cells Create Enhancers? A Biochemical Model of Enhancer Activation using Protein Trans-splicing**

Eva Parisi
Advisor: Tom Muir

Histone post-translational modifications (hPTMs) play a critical rule in the functional and structural organization of
chromatin. Increasing evidence suggests that aberrant gene expression patterns associated with cancer may result
from chromatin dysregulation at the hPTM level. Moreover, disruptions of the molecular machinery involved in
enhancer activation have also been implicated in several human cancers, as well as numerous developmental
diseases. Recent studies have identified two hPTMs –H3K4me1 and H3K27ac– as the epigenetic signature of active
enhancers. However, the specific sequence of events that creates this signature remains unknown, while available
technologies to investigate hPTMs in live cells are limited. Here, we hypothesize that H3K4me1 induces H3K27ac
deposition to activate genomic enhancers. First, we use an ultra-fast split intein to develop a protein trans-splicing
(PTS) technology for N-terminal protein modification in live cells. We then apply this method to semi-
synthesize the N-terminal tail of histone H3 with different PTMs at K4 and K27. We also report the first semi-synthesis of a
dually modified protein in live cells. We have thus established live cell PTS as a powerful chemical tool for precise
protein modification in biologically complex settings. Namely, we explore whether selective installation of H3K4me1
with PTS increases H3K27ac in live cells. We present preliminary data suggesting the epigenetic crosstalk of
interest may occur in trans in isolated nuclei. Future studies will require a more sensitive readout of local H3K27ac
levels to complement the global measurements performed in this work. Further characterization of the
relationship between H3K4me1 and H3K27ac will elucidate the exact mechanism of enhancer activation and
inform us of the molecular basis of many human diseases.

**Optogenetic Regulation of Branched-chain Higher Alcohol Biosynthesis to Control Advanced Biofuel Blends**

Olivia Parker
Advisor: Jose L. Avalos

With increasing greenhouse gas (GHG) emissions, the use of petroleum must be curtailed to avoid drastic climate
effects. The transportation sector currently accounts for 29% of GHG emissions in the United States and still relies
largely on petroleum-based fuels. * Biofuels, specifically branched-chain higher alcohols (BCHAs) such as isobutanol
(IbOH), isopentanol (IpOH), and 2-methyl-1-butanol (2-MBOH) are attractive alternatives. BCHAs are energy dense
and do not have the levels of volatility and hygroscopicity as their less energy dense counterpart, ethanol. The yeast
Saccharomyces cerevisiae, which naturally produces BCHAs in trace amounts can be metabolically engineered to
produce these compounds at higher quantities. This project’s aim is to develop genetically modified yeast strains
that have light inducible control of the BCHA pathways. This will allow for easier manipulation of the pathways’ titer
levels and a more efficient way to obtain the fuel with one strain in comparison to using two separate strains
simultaneously.
Investigating the Novel Mutation, rosette, and its Potential Function as a Region-specific Global Cue in the Core Planar Cell Polarity Pathway

Brooke Phillips
Advisor: Danelle Devenport

Morphogenesis, the shaping of an animal during development, relies in part on the Planar Cell Polarity (PCP) Pathway. PCP is defined as the coordinated organization of cell polarity across a tissue plane. Mutations in this pathway can disrupt developmental processes such as neural tube closure, inner ear and hair follicle patterning. While the core proteins involved in PCP have been well studied, the global cues that allow this pathway to regulate polarity across an entire tissue system, especially in mammals, remain elusive. A novel mutation, known as rosette (rst), causes a unique posterior-restricted hair follicle orientation defect in mice. Analysis of core PCP protein localization in the epidermis of rst mutants shows a maintenance of local polarity, but a loss of global polarity. This mutation’s ability to disrupt global polarity makes its potential function as a global cue promising. To elucidate rst’s possible interaction with known core PCP genes, Vang-Like 2 (Vangl2) and Frizzled6 (Fz6), heterozygous mutant animals were produced and screened for possible disruptions in hair follicle orientation, PCP protein localization and asymmetric polarization. I determined that the rst mutation genetically interacts with a Fz6 knockout (Fz6KO) mutation to produce phenotypes similar to those observed in rst homozygous mutants. Surprisingly, the rst mutation interacts with an overexpression (but not a dominant negative) mutation in Vangl2 to yield an even more dramatic phenotype. These genetic interactions suggest that rst plays a crucial role in balancing the levels of Fz6 and Vangl2 activity to coordinate PCP across the epidermis. Investigating this potential global cue could contribute to a better understanding of how global cues function in the core PCP pathway.

Investigating Poly (ADP-ribose) Polymerase 1 (PARP1) Epigenetic Crosstalks and Regulation by Oncohistone Mutations

Anagha Prasanna
Advisor: Tom Muir

Histone post-translational modifications (PTMs) are critical players in regulating various cellular processes. When this epigenetic machinery goes awry, it can dysregulate many molecular pathways to lead to human disease. One particular chromatin effector often implicated in cancer is Poly(ADP-ribose) polymerase 1 (PARP1), which is best known for its role in DNA damage repair. PARP1 adds ADP-ribosyl groups onto a host of substrates, including serine residues on histone H3. Recent studies have highlighted the interplay between histone ADP-ribosylation (ADPr) and other neighboring PTMs, better known as “crosstalks.” To further investigate the effect of local epigenetic and protein sequence contexts on PARP1 activity, this study assessed ADPr on reconstituted chromatin substrates containing modified residues or oncohistone mutants in vitro. These oncohistone mutants are potential drivers in cancer and inhibit chromatin effectors that directly interact with the mutated residue. They are suspected to affect other enzymes as well. Furthermore, epigenetic crosstalks involving ADPr were assessed in cells subject to DNA damage. As new oncohistones have been recently identified in patient tumors, a library screen was also performed to identify potential modulators of PARP1 activity. We found that phosphorylation at H3S10 and the mutant H3.3K9M significantly blocked PARP1 activity on histone substrates in vitro. Top hits from the oncohistone library screen also suggest that mutants important for DNA-histone contacts interfere with PARP1 activity, emphasizing the importance of DNA engagement for ADP-ribosylation. This work expands on the understanding of how PARP1 is regulated by chromatin context, both through PTMs and mutations, and how aberrations in its activity can be linked to oncogenesis.
An Investigation for Novel Cell Length Determinants in *Caulobacter crescentus* Reveals Unexpected Link to Metabolic Processes

Justin Ramos
Advisor: Zemer Gitai

Bacteria come in a diversity of cell shapes that facilitate their ability to thrive in a variety of environments. Specifically, the length of a given bacterium is important for maintaining a favorable surface area to volume ratio, which aids the bacterium in nutrient intake. Additionally, factors which regulate length serve as antibiotic targets because they are unique to bacteria. An excellent model organism to study cell shape is Caulobacter crescentus, an aquatic, rod-shaped bacterium. Caulobacter’s shape determinants have been the subject of research for many years. However, no study thus far has comprehensively surveyed all possible Caulobacter mutants for defects in length. In this thesis research, I performed a qualitative microscopy screen in a subset of an ordered transposon library being actively constructed to assemble a list of Caulobacter genes related to length. From the screen, I identified over fifty candidates with visible shape defects and confirmed them both phenotypically and genetically. I then attempted to characterize a cobalamin biosynthesis protein gene, confirming its role in the vitamin B12 biosynthesis pathway. Through this experiment, I also unexpectedly shed light on a lesser-known cell growth paradigm, which unites cell growth, shape, and metabolism in active growth regulation during prolonged stationary phase. Many of the identified factors are uncharacterized and conserved among other Gram-negative bacteria. Their conservation indicates they may play a fundamental role in cell elongation, division, and life cycle regulation. These novel factors also provide an opportunity to elucidate the full mechanism of shape determination and grant insight into new antibiotic targets.

Elucidating the Role of Heparin in Fibronectin Module III-13 Self-Association

Ellie Randolph
Advisor: Jean E. Schwarzbauer

The extracellular matrix (ECM) creates a dynamic 3D environment for cells, providing both mechanical and biochemical structure. Proper development of the ECM is dependent on the initial formation of a fibronectin (FN) matrix, and other matrix components depend on this FN matrix for ECM incorporation. FN matrix assembly is facilitated by unfolding of the compact FN dimer and which exposes hidden binding sites along FN. FN-FN interactions form fibrils, and over time these fibrils are converted into an insoluble form. We identified one type III module of FN, III-13, as important for formation of insoluble fibrils. III-13 not only demonstrates structural features that permit self-association, but it also lies in the center of FN’s heparin-binding (HepII) domain, and heparin was recently found to promote formation of the FN matrix. We hypothesized that heparin binding could be related to self-association. To test ability of III-13 to self-associate, we measured the thermal unfolding of III-13 by fluorimetry and used dynamic light scattering to detect different size populations that III-13 can form, both with and without the addition of heparin. We discovered that when heated III-13 experiences a conformational change before fully denaturing, and that this conformational change facilitates the formation of III-13 multimers. Heparin binding to III-13 increased the temperature needed to reach the conformational change, indicating that heparin stabilizes III-13, but with increasing temperature III-13 formed multimers and aggregates. Because one heparin chain can bind multiple III-13 modules, a model is proposed in which heparin brings III-13 monomers together, increasing the chance that they will form connections as they partially unfold. Although FN matrix formation does not occur at high temperatures in vivo, we discuss other factors that can induce III-13 unfolding and how heparin assists in the order and organization of this process.
Dendritic Disasters: Investigating the Role of Muscleblind (mbl) in Drosophila class IV Dendritic Arborization Neurons
Denay Richards
Advisor: Elizabeth Gavis

The ability of an organism to respond to its environment relies on effective signal transduction in neurons of the peripheral nervous system (PNS). As a result, the organization of the dendritic arbor in multidendritic PNS neurons must be tightly regulated. Our lab has shown that the Drosophila melanogaster RNA-binding protein, Muscleblind (Mbl), is important for the formation of the dendritic arbor, in class IV dendritic arborization (da) neurons – multidendritic neurons of the Drosophila PNS that function in nociception. This thesis seeks to further understand the morphological defects associated with mbl knockdown, identify the molecular and cytoskeletal disruptions caused by Mbl reduction, observe the impact that these defects have on larval nociception and propose a model of Mbl function. Ultimately, I find that mbl knockdown causes an increase in terminal branch density and a decrease in field coverage – the ability for the dendritic arbor to cover the epidermal space. However, the percentage of the arbor that invaginates into the epidermis, a process termed enclosure, remains unchanged between the wild-type and mbl RNAi class IV da neurons. In terms of cytoskeletal organization, mbl RNAi class IV da neurons show a decrease in stable microtubules. Overall, these defects result in larval hypersensitivity to global thermal stimulus but no impact on mechanical nociception. It is our hope that these data can be used to better understand the role of Mbl, and its human homolog muscle-blind like protein (MBNL) in regulating mRNA stability and preventing disorders like Myotonic Dystrophy (MD), a genetic condition in humans mutant for MBNL.

Genetic and Biochemical Analysis of MAB21 Proteins in Neuroblastoma Proliferation
Joseph Ryu
Advisor: Chandra L. Theesfeld

Neuroblastoma is the most prevalent and deadly form of childhood cancer with limited treatment options. To facilitate study of this rare disease and hundreds of others, we have developed a novel computational approach, known as URSAHD, that compares gene expression signatures of one disease to over 300 other diseases and normal tissues. The URSAHD neuroblastoma model identified MAB21L1 and MAB21L2 as unique to neuroblastoma. MAB21 proteins play a critical role in limb and nervous tissue development across animal models and humans, but there is no documented link between MAB21 proteins and neuroblastoma, nor is there clear evidence that MAB21 proteins have a catalytic function. Extending on preliminary results, this study finds that reduced levels of MAB21L1 and elevated levels of MAB21L2 both generate a growth defect. However, they do not interact in a synergistic manner, hinting that the MAB21 homologs do not impact growth together. Proteins assays reveal that native MAB21L1 and MAB21L2 preferentially localize in the nucleus and cytoplasm, respectively. In sum, our results suggest that MAB21L1 promotes cell growth in the nucleus and MAB21L2 perturbs cell growth in the cytoplasm. Notably, the MAB21L1 antibody cross-reacts with MAB21L2, revealing a way to measure MAB21L2 expression. Through this study, we offer the first in vivo functional assay for MAB21L1 and MAB21L2 in neuroblastoma cells, opening the door for deeper mechanistic and pathway-level analyses of these highly conserved proteins with great importance to human nervous system development and disease.
**Reannotator: A Tool for Comparative Analysis of RNA-Seq and its Application in Highlighting Differences Between HCMV and Oncoviruses**

Yoni Schoenberg  
Advisor: Thomas Shenk

RNA-sequencing technology (RNA-seq) allows for measurement of mRNA in cells, providing gene expression levels. RNA-seq data from published studies are available in a public repository maintained by the National Center for Biotechnology Information (NCBI), but the metadata is difficult to access, making cross-study analyses challenging. Here we describe a tool that we developed, which we have named Reannotator, that organizes RNA-seq metadata into an editable spreadsheet format. Metadata can be viewed and experimental conditions annotated into specific columns, allowing for downstream analysis of the RNA-seq data for differential gene expression levels between experimental conditions. To show the utility of our tool, we use it to analyze RNA-seq data from cells infected with different viruses. We explore expression levels of p53 target genes in cells infected with different known oncoviruses and Human cytomegalovirus (HCMV), which all inhibit activity of the p53 tumor suppressor, to better understand why HCMV has not been shown to be an oncovirus. Our results identify three genes or family of genes that are upregulated in HCMV-infected cells and downregulated in cells infected with oncoviruses. These findings highlight potential novel gene functions that can be tested in the lab, which ultimately could lead to understanding how to mitigate the effects of p53 mutations in cancer. Additionally, they highlight our tool’s ability to help answer many questions in biology using publicly available RNA-seq data.

**Characterizing Outer Membrane Protein Biogenesis in *Escherichia coli* using a Small-molecule Inhibitor of BamA and its Resistant Mutation**

Jessica Sheng  
Advisor: Thomas J. Silhavy

Gram-negative bacteria are especially resistant to antibiotics due to the presence of an additional layer to the cell envelope called the outer membrane. Outer membrane proteins (OMPs) are transmembrane proteins found in this outer membrane, and their synthesis and assembly are of great research interest, as disrupted OMP assembly causes cell death and could be a target for new antibiotics. The β-barrel assembly machinery (Bam) complex, which is composed of five subunits (BamA, B, C, D, and E), takes unfolded OMPs and assembles them for insertion into the outer membrane. The exact mechanism of this assembly is still not completely understood. The primary goal of this study was to identify compounds that target the Bam complex, as their interaction with the Bam complex could help better characterize the Bam mechanism and lead to novel antibiotic development. We conducted screens with small-molecule compounds that showed potential in disrupting OMP biogenesis. The compound TJS-300 killed Gram-negative bacteria that possessed functional efflux pumps and demonstrated increased potency against strains with disrupted OMP assembly, suggesting that it acts by interacting with and inhibiting BamA on the outer membrane surface. A resistant mutation to TJS-300, bamAE470K, does not alter BamA levels or cause activation of the σE stress response, suggesting that it causes only a conformational change in BamA and does not confer resistance via any change in function. Thus, TJS-300 most likely directly inhibits BamA on the outer membrane surface. This study contributes to our understanding of the mechanism of OMP biogenesis and to development of potential antibiotics that target this pathway.
Characterizing S. cerevisiae Pif1 Residues: Identifying the Nuclear Localization Signal and Testing in vivo Functions of Residues Associated with G-quadruplex DNA

Ashley Stone
Advisor: Virginia Zakian

Maintaining genome integrity is a critical function of cells. Pif1, a multifunctional DNA helicase found in both the nucleus and mitochondria, promotes genome integrity. In this two-part study, mutational analysis was conducted to identify residues that are essential for Pif1 functions. In the first part, the previously undiscovered nuclear localization signal (NLS) of Pif1 was identified. Residues 781-Lys-Lys-Arg-Lys-784 were predicted to be the NLS through computational analysis and literature review. Cells with the pif1-781-784Δ allele displayed disrupted nuclear Pif1 functions, including Okazaki fragment (OF) maturation and telomerase inhibition, but preserved mitochondrial DNA (mtDNA) maintenance function. The functional NLS was then confirmed to be residues 781-784, because the phenotype of pif1-781-784Δ was rescued by the addition of the SV40 NLS sequence. The pif1-781-784Δ allele is a valuable experimental tool that allows researchers to explore the effects of the absence of Pif1 in the nucleus. In the second part of this study, the unwinding of G-quadruplex (G4) DNA by Pif1 was investigated in vivo. G4 DNA are secondary structures that result from noncanonical base-pairing, and a recent in vitro study identified mutations that reduce the ability of Pif1 to bind and unwind G4 DNA. The effects of these mutations on in vivo Pif1 functions were tested and showed that they disrupt OF maturation, but not mtDNA maintenance. As G4 structures impede fork progression and induce damage, elucidating the mechanisms of G4 unwinding by Pif1 is important to understanding the promotion of genome integrity.

A “Complex” Problem: Leveraging Spatial and Temporal Information to Investigate Dynamic Protein-protein Interactions

Samvida Sudheesh Venkatesh
Advisor: Ileana Cristea

Large omic datasets with dynamic characterizations of biomolecules across space and time hold immense potential to provide valuable insights into biological processes. However, parsing this information poses significant challenges in the interpretation of multidimensional data. The field of protein-protein interactions would especially benefit from advancements in visualizing, analyzing, and integrating spatially and temporally dynamic information. To address this need, I developed VISTA (Visualizing Interactions in Space and Time Analysis tool), a computational program to explore dynamic protein interaction networks through animated visuals and network reports, with automatic integration of published knowledge on protein localizations, functions, and interactions. The program is freely available for download at https://github.com/samvidav/VISTA. To demonstrate the utility of VISTA, I performed immunoprecipitation and mass spectrometry analyses of three viral proteins of the spatially and temporally regulated pathogen human cytomegalovirus (HCMV) and used the integrated analysis capabilities of VISTA to better define their biological roles. Early in infection, pUL13 interacts with actin remodeling proteins, potentially contributing to cellular rounding, while at later timepoints, pUL37 and pUL13 likely have cooperative roles in the mitochondria to disrupt mitochondrial morphology through the MICOS complex. pUL13 additionally interacts with proteins in the unfolded protein response pathway in the virus assembly complex. Finally, a preliminary investigation also identified interactors of pUL82 involved in viral gene expression and cell cycle control. In Chapter 2, I expanded upon VISTA bioinformatic analysis of proteomics data to infer protein complex conservation and divergence in the heart proteomes of four cardiac model organisms, uncovering species-specific immune and metabolic protein complexes, as well as conserved roles for protein expression and localization in all
species studied. Overall, this thesis synthesizes a range of computational and experimental methods for the study of protein interactions in diverse biological contexts and extends these tools to other researchers for broad application in the field.

Repressors of Quorum-Sensing-Induced Aggregation in Vibrio Cholerae

Dominique Summerville
Advisor: Bonnie Bassler

Vibrio cholerae is the pathogen responsible for the disease cholera. To cause disease, V. cholerae must have the ability to form surface-attached multicellular communities called biofilms. The formation of surface biofilms is regulated by quorum sensing (QS). QS is a process in which bacteria collectively regulate gene expression in response to the secretion, accumulation, and detection of signaling molecules called autoinducers. The low cell density QS-state promotes biofilm formation while the high cell density QS-state promotes biofilm dispersal.

Previous work in the Bassler laboratory identified an aggregation program in V. cholerae that occurs in liquid in the high cell density QS-state and is independent of components known to be required for surface biofilm formation. In this previous work, members of the Bassler group identified genes that function as activators of aggregation. In this study, I identified putative repressors of aggregation by performing a transposon mutagenesis screen in a low cell density-locked QS-state V. cholerae strain, a genetic background in which cells are normally unable to aggregate. This screen was successful, and I discovered two genes that act as repressors of aggregation: fbp and clpX. I validated both fbp and clpX and eliminated some mechanisms by which these genes may be regulating aggregation, which included control of the levels of O1 antigen and modulation of the QS-state of the cells. These findings increase our understanding of mechanisms that V. cholerae uses to transition between the planktonic and community phases of its life cycle.

Evolution of Genomic Imprinting: Re-Visiting the Kinship Theory

Yared Tamiru
Advisor: Shirley Tilghman

Genomic imprinting describes the monoallelic expression of specific loci based on parent of origin of the allele. The prevailing theory on the evolution of imprinting is known as the “Kinship Theory,” which postulates that imprinting is the result of a conflict between the paternal and maternal genomes over the growth and development of the embryo. However, this theory, like many others, was based upon the very small sample size of known imprinted genes in mammals (~126 in mice). In 2005, Leudi et al. claimed to have identified ~600 new imprinted mouse genes using machine learning classifiers based on sequence data. The goal of this thesis was to rigorously examine which of these mouse genes identified by Leudi et. al are truly imprinted, and attempt to reconcile their molecular functions and parental expression bias with the kinship theory. Upon filtering the Leudi et al. genes based on more stringent criteria for imprinting, 6 new imprinted clusters were identified and mapped. Subsequent GO analysis surprisingly showed a strong enrichment of GO terms associated with neurological development. I conclude that these results are, in fact, consistent with the Kinship Theory because behaviors during infancy are critical to an offspring’s growth. Therefore, the genes underlying them are mediated by imprinting in the same way genes underlying fetal growth are mediated. In this way, the Kinship Theory’s intragenomic conflict extends itself beyond nutrient allocation in utero; it mediates imprinting after birth, when the infant is still dependent on its mother.
Investigating Sirtuin-3 in Host Defense Against HCMV Infection
Amy Tien
Advisor: Ileana Cristea

Successful viral infection relies upon virus modulation of host cell processes for both progeny replication and immune response evasion. In human cytomegalovirus (HCMV) infection, impacted processes include altered regulation of metabolic pathways and mitochondrial biogenesis and respiration, as well as morphological disruption. Recent work has found NAD+-dependent deacetylase sirtuin-3 (SIRT3) to act in host defense against RNA and DNA viruses, including HCMV, though the mechanism through which this is accomplished remains unknown. Given the well-established role of SIRT3 in metabolic regulation, this thesis investigated the hypothesis that SIRT3 assists antiviral response via counter-regulation of host factors in pathways modulated by viral infection. Characterization of SIRT3 interactions at different stages of infection was conducted using an immunoaffinity purification (IP)-mass spectrometry (MS)-based methodology to analyze infected cells with overexpression of tagged SIRT3. Functional classification of identified interactors emphasized oxidative stress response, mitochondrial protein synthesis, ATP synthesis, the TCA cycle, glucose metabolism, amino acid metabolism, and fatty acid metabolism as key cellular processes of SIRT3 and viral association. Viral titer assays of infected nigericin-pretreated cells additionally highlighted regulation of mitochondrial membrane potential as a pathway of interest, supported by the presence of ATP synthase interactions. Comparison of interaction networks across time points of infection indicate the role of SIRT3 to be temporally dynamic, with most enhanced associations during early infection at 24 hpi. Furthermore, IP-MS uncovered compelling interactors found to be of interest due to strong changes in interaction abundance compared to mock at every stage of infection. The role of deacetylase activity in SIRT3 antiviral function was studied through comparison of interaction abundance findings and previously established acetylation data for known substrates. Results suggest the presence of strong deacetylase function during early infection that is attenuated as infection progresses. This substrate comparison was supported by visualized inhibition of mitochondrial fragmentation by catalytically active SIRT3 in contrast to a catalytically inactive mutant. Taken altogether, the findings of this thesis begin to characterize SIRT3 function in host defense as an early mechanism mediated through deacetylase activity in identified mitochondrial pathways. Continuation of this initial work will further elucidate the underlying mechanism behind the antiviral role of SIRT3 to hopefully assist in the design of novel HCMV therapies.

Investigation of the Fog Signaling Pathway
Annan Timon
Advisor: Jared Toettcher

In the complex and dynamic environment of the early embryo, individual cells must first receive signals in order to initiate the process of tissue folding. These signals occur at specific locations and at exact times during the organism’s developmental timeline. Here, we examine the secreted autocrine signaling protein Folded gastrulation (Fog) and its receptor the mesoderm-invagination signal transducer (Mist). In this thesis we set out to simultaneously control both Fog and Mist expression so that we could test whether their co-expression is sufficient for tissue contractility. We took two parallel approaches: directly inducing Fog and Mist expression using the GAL4-UAS system, and developing an optogenetic expression system so that their expression could be controlled more precisely in space. We find that while Fog and Mist are individually necessary to induce these cellular movements, their co-expression is not sufficient to reproduce the contractility driven by optogenetic Erk activation. The work presented here helps to define the roles of Fog and Mist in the early embryo and tests the efficacy of our first-generation Opto-GAL4 system.
Curcumin, a potent antioxidant and anti-inflammatory compound found in the curry ingredient turmeric, has been shown in vitro and in animal models of Alzheimer’s disease (AD) to reverse neurodegeneration and cognitive decline. However, the low bioavailability of curcumin poses a challenge to its potential neuroprotection in humans. Clinical trials have recently been published to test whether curcumin reliably alleviates or prevents AD-associated cognitive decline, providing a valuable opportunity to investigate emerging patterns from the current literature. Hence, this thesis presents a meta-analysis of these five studies meeting inclusion criteria and tests for curcumin’s overall and differential effects on subgroups based on participant characteristics, curcumin dosage, and intervention duration. Using cognitive task performance as the primary outcome of interest, meta-analyses did not show a statistically significant effect of curcumin overall or in any of the subgroups assessed. However, there was a trend toward a positive effect favoring curcumin’s neuroprotective effects on non-demented adults. Additional studies with larger sample sizes and consistent measurement of plasma curcumin can provide more statistical power to determine whether bioavailable curcumin serves a robust neuroprotective role in humans. Nevertheless, regular consumption of turmeric along with ingredients such as black pepper and cumin seeds, which enhance curcumin’s bioavailability and additionally serve gastroprotective roles, may produce synergistic neuroprotective effects that optimally outweigh those of curcumin in isolation. In collaboration with Princeton University Campus Dining, tentative recipes incorporating these ingredients were thus created as part of an evidence-based, holistic approach toward a neuroprotective diet.

Localizing the Most Highly Conserved Proteins in Photosynthetic Organisms
Kelly Van Baalen
Advisor: Martin Jonikas

Global food demand is projected to double by 2050. To meet this increased demand without causing further environmental degradation through land conversion, crop yields must be increased on existing agricultural land. One promising route for increasing yields is to genetically modify crop plants to have greater photosynthetic efficiency. Progress has already been made in improving the photosynthetic efficiency of some plants through genetic editing and more significant increases are possible through more complex editing. However, such improvements are constrained by the fact that the identities and specific functions of many genes with putative roles in photosynthesis are currently unknown. The GreenCut2 list of proteins, which are conserved in photosynthetic organisms, provides many promising candidates for involvement in photosynthetic function. Using a high throughput fluorescence tagging method, we determined the localizations of 53 GreenCut2 proteins in the model alga, Chlamydomonas reinhardtii. 27 of these proteins were found to localize to the chloroplast, 20 of which were not previously known to be in the chloroplast proteome. Our results expand the known list of photosynthesis related proteins and provide valuable clues as to their function, improving our understanding of photosynthesis and enabling improved genetic engineering of crop plants.
Neural tube closure in *Ciona intestinalis*: Investigating the Gene Regulatory Network of *Lmx*

Nancy Wenger
Advisor: Michael Levine

Neural tube formation is an intricate process that requires proper regulation of cell division, movement, and shape changes. It is influenced by both environmental cues and genetic factors. Perturbation of its closure leads to neural tube defects (NTD’s) such as spina bifida. However, a comprehensive gene regulatory network has yet to be fully elucidated due to the complexity of vertebrate systems. *Ciona intestinalis* serves as a model organism for the purpose of this research because of its highly simplified neural tube and fixed embryonic cell lineage. The transcription factor *Lmx*, expressed in the dorsal neural tube cells in *Ciona*, has shown an intercalation defect when down-regulated. To gain more insight into the role for *Lmx* during neural tube formation, I ectopically expressed the gene in the epidermis adjacent to the neural plate. *Lmx* overexpression in this region results in dorsal curvature of the tail instead of ventral, and in most cases, the tube fails to close. The overexpression of *Lmx* did not affect the epidermal identity of the cells in triggering them to adopt a neural fate. In a single cell transcriptomic screen of *Lmx* overexpressing embryos, *Lmx*, *DCDC2*, and *PIM3* expression were affected by the *Lmx* misexpression. Fluorescence intensity quantifications of reporter gene expression suggested *Lmx* activation of itself and inhibition of *DCDC2* and *PIM3*, although results were not statistically significant and thus failed to conclusively confirm their regulation by *Lmx*.

Functional Analysis of *germ cell less* During Germ Cell Formation and Specification in *Drosophila melanogaster* Embryos

Lillian Wilkins
Advisor: Girish Deshpande

Transcriptional quiescence is an evolutionarily conserved trait that distinguishes the embryonic Primordial Germ Cells (PGCs) from their somatic neighbors. In *Drosophila melanogaster* embryos, *Germ cell less* (*gcl*) protein is required for proper pole cell formation and the establishment and maintenance of transcriptional quiescence in PGCs. Thus, PGCs from *germ cell less* (*gcl*) embryos ectopically express several somatic genes including two X-linked numerator elements, *sisterless-A* (*sisA*) and *sisterless-B* (*sisB*). These two proteins are shown to activate transcription of *Sex-lethal* (*Sxl*), a sex-determination gene that orchestrates female identity in the somatic nuclei. Importantly, wild type (WT) pole cells from blastoderm stage embryos do not express *Sxl* and are naïve with respect to their sexual identity. Here, we have examined *Sxl* expression in *gcl* PGCs. Consistent with precocious activation of numerator elements, *Sxl* is inappropriately activated on a transcriptional level and in a sex-nonspecific manner in *gcl* PGCs. Reciprocally, ectopic expression of *gcl* in the soma is sufficient to inhibit *Sxl* expression. Precocious expression of *Sxl* in PGCs results in consistent reduction in the number of PGCs in early embryos and disrupts their migration during mid-embryogenesis. Supporting the conclusion that *Sxl* is a critical target of Gcl, simultaneous removal of *Sxl* and *gcl* mitigates the loss of PGCs observed in *gcl* embryos. These observations underscore the biological relevance of transcriptional quiescence in the embryonic PGCs and establish *Sxl*, the master determinant of female somatic fate, as a critical target of silencing mechanisms in PGCs. Finally, considering the findings of two recent papers, we propose two possible models for the activity initiated by Gcl.
The Antimicrobial Role of Candidate Biosynthetic Gene Clusters from *Actinomyces* Strains within the Oral Human Microbiome

Lucy Williamson
Advisor: Mohamed Abou Donia

The oral community is an essential part of the human microbiome controlled by both aerotolerant and anaerobic commensal bacteria including the Streptococci and Actinomyces genera. Recent studies have shown the antimicrobial role of commensals through production of antibiotic metabolites that can inhibit bacterial growth within the human microbiome. These secondary metabolites are produced through biosynthetic pathways regulated by biosynthetic gene clusters (BGCs), which have become a key genomic tool in identifying candidate gene clusters to test for antibiotic bioactivity. Two BGCs – a phenazine-like cluster and a thiopeptide-like cluster – from different Actinomyces spp. were identified using an antiSMASH similarity analysis against known homologous antimicrobial gene clusters. We hypothesized that these BGCs each produce secondary metabolites that can inhibit both pathogenic and commensal growth within the oral community. We endogenously cultured BGCs within their Actinomyces strains to assess the cell extracts using 1) bioassays against a panel of oral indicator pathogens and commensals to test for antimicrobial activity, and 2) liquid chromatography-mass spectrometry (LC-MS) to analyze extract composition for phenazine-like and thiopeptide-like molecules. The bioassays have shown potential bioactivity at lower extract concentrations, but heterologous expression of the phenazine BGC in E. coli BL-21 has shown little evidence of phenazine in comparative LC-MS analyses. Further experimentation into endogenous Actinomyces LC-MS analysis and the heterologous expression of the thiopeptide candidate BGC could help to determine the importance of Actinomyces abundance in the human microbiome for potentially limiting both pathogenic and commensal growth within the oral community.

Development of an Experimental Paradigm for the Investigation of Innate Immune Pathways Restricting Hepatitis Delta Virus Replication

Evelyn Wu
Advisor: Alexander Ploss

HDV is a single-stranded, negative-sense RNA satellite virus, dependent on HBV envelope proteins to complete its replication cycle. HBV/HDV co-infection increases the likelihood, severity, and rate of liver disease onset. HDV can only establish robust infection in humans and chimpanzees, which poses a challenge to studying immune responses to HDV and assessing the efficacy of antiviral treatments. The discovery of the human sodium co-transporting polypeptide (hNTCP) as the functional receptor for HBV and HDV has helped overcome the barrier to entry in non-susceptible cells and has led to the generation of various in vitro and in vivo models. However, work remains to be done to develop a small animal model that accurately recapitulates HDV pathogenesis. To better understand HDV viral-host interactions, we first established a system for studying immune responses to HDV independently of HBV infection in vivo. We showed that supplying HBV surface proteins (HBsAg) in trans via an AAV vector supported HDV infection in both liver chimeric mice and a mouse model that transgenically expresses hNTCP on a bacterial artificial chromosome (NRG-hNTCP/BAC). Using this system, we observed that HDV persisted in engrafted human hepatocytes of liver chimeric mice for at least 8 weeks while it was cleared in the hepatocytes of our NRG-hNTCP/BAC model under mono-infection conditions. To study the mechanisms of the observed difference in persistence, we generated knockouts of innate immune pathways that could restrict HDV replication in murine hepatocytes by using recombinant AAV to deliver CRISPR/Cas9 components to NRG-hNTCP/BAC mice. Greater HDV RNA copy numbers in NRG-hNTCP/BAC mice that harbored a partial knockout of MAVS suggests a role for RIG-I-like receptor (RLR) signaling in HDV clearance. However, the challenge of generating a homogenous, efficient knockdown in vivo precludes any definitive conclusions and remains to be resolved. The findings in this study contribute to establishing an experimental framework for studying innate immune pathways that might be
involved in antagonizing HDV replication in murine hepatocytes. Identification of such pathways would contribute to creating a better small animal model that recapitulates natural HDV infection, identifying a point of intervention for treatment, and a better understanding of the immune response to the virus.

Identifying the Subcellular Localization of Poorly Characterized Proteins Required for Photosynthesis
Yihua Xie
Advisor: Martin Jonikas

Photosynthesis is essential to all living beings on Earth, providing most of the food, energy, and material. Developing a deeper understanding of photosynthesis would give us tools to improve plant yield, which has become an urgent desire due to the soaring food demands from a rapidly expanding population. Despite the importance of photosynthesis, many underlying mechanisms and proteins remained enigmatic. In order to advance our understandings, we utilize a fluorescent protein tagging pipeline and a gene rescue approach in the model alga Chlamydomonas reinhardtii to determine the localization and function of a group of poorly characterized phototrophic growth-related genes. Our data reveal the subcellular localization of 14 proteins and demonstrate 8 genes’ involvement in photosynthesis, shedding light on these once obscured genes and pathways and contributing to new opportunities for engineering photosynthesis that were previously unavailable.