Malaria in Pregnant Women and Children in Ghana

Aseel A. Abdalla

Adviser: Adel Mahmoud

Despite persistent eradication and control strategies, malaria remains one of the deadliest parasitic diseases. While malaria affects nearly 500 million people annually, pregnant women and young children appear to be uniquely susceptible to the disease. Pregnant women living in high transmission settings tend to suffer severe maternal anemia leading to low infant birth weight due to preterm delivery or intrauterine growth restriction. Many cases of pregnancy-associated malaria end in either the death of the mother or her fetus. Malarial infections in non-immune children often result in severe anemia, respiratory distress or even cerebral malaria. In malaria-endemic regions, immune protection is accompanied by increased levels of antibodies capable of binding parasitized red blood cells. These antibodies recognize variant surface antigens presented on the surface of the red blood cells by mature forms of the parasite. This investigation assesses the antibody response to malarial infection among residents of Asutsuare, a Ghanaian village where P. falciparum transmission is particularly high. To do so, this study measured the antibody levels in serum samples collected from pregnant women, children, and adults in this community using indirect immunofluorescence. The results of this investigation improve our understanding of acquired immunity to P. falciparum in high transmission settings and highlight the complex host-parasite interactions that take place during the course of malarial infection.

The impact of sirtuins on mitochondrial and cytoplasmic organization during HCMV infection

Yaa Abu

Adviser: Ileana M. Cristea

Sirtuins (SIRTs) are a family of seven NAD+ dependent protein deacetylases that regulate gene expression and control epigenetic modifications of proteins. Though the role of SIRTs in cancer has been extensively researched, recent work suggests SIRTs also have antiviral properties, though the mechanism for their antiviral activity remains unknown. Thus, the objective of this study is to investigate the mechanism through which SIRTs act during infection, with a focus on potential interactions with binding partners and changes in enzymatic activity during human cytomegalovirus (HCMV) infection. Specifically, this study uses SIRT2, which deacetylates lysine residues in the cytoplasm, to examine changes in cytoplasmic organization during viral infection. Currently, I have optimized isolation of Flag-SIRT2 using a-Flag antibody and will use co-immunoprecipitation (CO-IP) and IP with tandem mass spectrometry (IP/MS-MS) to validate known and potential interactions of SIRT2. To determine whether SIRT2s antiviral functions are dependent on its enzymatic activity, I have also chosen, through Western analysis, an appropriate time point through which SIRT2 inhibitor AK7 inhibits SIRT2 deacetylase activity. Future experiments will examine SIRT4, which regulates the mitochondrial pyruvate dehydrogenase (PDH) complex as a cellular lipoamidase, to further examine how viral infection influences mitochondrial organization. Ultimately, elucidating the mechanism through which SIRT2 and 4 inhibit viral replication could lead to identification of new targets for therapeutics against HCMV.

Role of BamA Conformational Changes in the Mechanism of Outer Membrane Protein Assembly in *Escherichia coli*

Funmi Adetunji
Anne McCabe
Adviser: Thomas Silhavy

The outer membrane of Gram-negative bacteria serves as a selective permeability barrier by keeping toxic compounds out of the cell, while allowing nutrients to enter. Outer membrane β-barrel proteins (OMPs) perform crucial functions in helping the cell adapt to its environment, yet the mechanism for their assembly is not well understood. In Gram-negative bacteria, OMPs are assembled by the β-barrel assembly machine (Bam) complex. The essential components of the Bam complex are the OMP BamA, which contains an amino-terminal periplasmic extension composed of five polypeptide transport-associated (POTRA) domains, and the lipoprotein BamD. In *Escherichia coli*, the Bam complex also contains three nonessential lipoproteins, BamBCE, all of which require the POTRA 5 domain for stable interaction with BamA. Studies have shown the presence of conformational changes within the well-conserved region POTRA 5 domain critical for interaction with BamD and β-barrel assembly *in vivo*. Mutations known to affect BamA function influence local POTRA 5 dynamics, suggesting a critical role for POTRA 5 plasticity in OMP folding and insertion. POTRA 5 mutations affecting the interaction between the Bam subcomplexes provide useful genetic tools for analyzing the OMP assembly process. We report the characterization of mutations in residue K351 of POTRA 5 that alter the electrostatic network involving E373 and R366, residues important for BamA-BamD interaction and OMP assembly. The mutation *bamAK351E* suppresses the lethal mutation *bamAE373K* and suppresses the growth and permeability defects of *bamDR197L bamAR366A*. The fact that K351E suppresses the OMP assembly defects of POTRA 5 mutations that compromise the interaction with BamD suggest that K351E restores the interaction with BamD. These results highlight the importance of BamA-BamD interaction for OMP assembly. Biochemical analysis of BamA conformational dynamics, Bam complex stability, and outer membrane permeability in newly identified POTRA 5 mutants can provide insight into the mechanism of OMP assembly.

The Structure and Function of the SM Protein Vps45

Frederick Allen
Adviser: Frederick Hughson

Vesicle fusion in eukaryotes is dependent on vesicle-SNAREs (v-SNAREs) and target membrane SNAREs (t-SNAREs) forming heterotetramers, and SNARE activity is regulated by Sec1/Munc18 (SM) proteins. SM proteins have been shown to interact with both v-SNAREs and t-SNAREs; the structural basis for an interaction between the SM protein Vps33 and its v-SNARE Nyv1 led to the hypothesis that SM proteins facilitate *trans*-SNARE complex formation. Understanding the thermodynamics and structural basis for other SM/v-SNARE interactions should indicate whether this hypothesis is unique to Vps33 or is universal for SM proteins. One such interaction that has not been extensively studied is between the SM protein Vps45 and the v-SNARE Snc2. Gel filtration chromatography suggests that Snc2 does not bind Vps45; isothermal titration calorimetry will be used to confirm this, and X-ray crystallography to visualize any interaction, or Vps45 alone. It is hypothesized that under certain conditions an interaction exists between Vps45 and Snc2 resembling that of Vps33 and Nyv1 thermodynamically as indicated by similar K_d and structurally as indicated by the orientation and placement of the v-SNARE on the SM protein. Confirming this hypothesis would indicate that SM proteins share a universal mechanism of SNARE regulation through v-SNARE interactions.

Investigating the Anterior-Posterior Extent of the Rat Posterior Parietal Cortex

Cecilia Barowski

Adviser: Carlos Brody

Neuroscience studies in the past decade have largely focused on gaining a better understanding of the neural mechanisms underlying decision-making and attention. Functional components of attention have been modeled to incorporate how various types of information is processed and how information is translated across different brain regions; imaging studies have identified areas with interacting pathways that are consistently activated during tasks regarding attention. One such region is the posterior parietal cortex (PPC). Using retrograde tracers (florescent dyes which travel backwards along neurons) injected directly into the rat brain, we hope to elucidate the anterior-posterior boundary of the PPC. The PPC receives signals from surrounding regions, while the regions directly surrounding those receive signals from different regions. As we incrementally move the retrograde tracer injection sites along a line running parallel to the anterior-posterior axis within the PPC, we expect to see only the regions which signal the PPC directly to receive tracers. If additional regions receive tracers, then the anterior-posterior boundary of the PPC has been crossed. We hope to gain results using two retrograde tracers showing two injection sites straddling this border indicating the bounds of the PPC.

Suppressing quorum sensing in *Staphylococcus aureus* using flow and non-cognate auto-inducing peptides: a research plan using microfluidic approaches

Rachel Bergman

Adviser: Howard Stone

Staphylococcus aureus (*S. aureus*) is a major cause of many nosocomial infections. Over the last 70 years, different strains of the bacteria have evolved resistance to several different antibiotics, making continued development of new antibiotics necessary for treatment. As such, other potential therapeutics have been explored, including suppressing the ability of *S. aureus* to perform quorum sensing (QS). One method of suppressing QS is to use antagonistic auto-inducing peptides (AIPs), the signaling molecule that exists in distinct forms for different strains of *S. aureus* and inhibits the signaling pathway of non-cognate strains. Previous studies have shown that fluid flow can also inhibit QS by advection of signaling molecules, thus preventing activation of QS signaling pathways.

I intend to use microfluidic approaches and confocal microscopy to study the extent to which non-cognate AIPs and fluid flow can suppress QS of *S. aureus* in a co-culture with human epithelial cells. Preliminary results show that both flow and antagonistic AIPs can inhibit QS, but that it is difficult for flow and molecules to reach all the crevices in corrugated geometries, such as those seen of *in vivo* tissues like the gastrointestinal tract. By performing flow experiments in a co-culture with human epithelial cell and using a corrugated chamber design, my research will highlight if the physiological conditions of an *in vivo* system, such as pH and geometry, diminish the effect that non-cognate AIPs and fluid flow have on suppressing QS.

Single-cell transcriptomic characterization of *Plasmodium falciparum* and *P. vivax* liver stage infections in human liver chimeric mice

Michael Chang
Adviser: Alexander Ploss

Malaria remains a global health concern, causing over 600,000 deaths annually, and its two most virulent strains, *Plasmodium falciparum* and *Plasmodium vivax*, place almost 3 billion people at risk for the disease. Insufficient knowledge of host-parasite interactions has hindered development of effective therapies. Human liver chimeric mice, or mice engrafted with human hepatocytes, have been established as a physiologically relevant model for studying liver disease, including *P. falciparum* malaria. Using humanized mice previously developed by the Ploss lab, this study aims to characterize the transcriptomic profiles underlying *P. falciparum* and *P. vivax* stage infections in the human liver. Such liver chimeric mice will first be infected with *P. falciparum* and *P. vivax* sporozoites through direct feeding and fresh sporozoite injection. Sporozoites will be traced *in vivo* during liver development via DiR fluorescent dye and isolated from human liver sections using laser capture microdissection. RT-qPCR and immunofluorescence microscopy will then be used to quantify and visualize parasitemia in the extracted sections. Preliminary work to optimize the staining and injection process, in addition to RT-qPCR and immunofluorescence of extracted liver tissue, has been promising for our future experimentation with fresh *Plasmodial* sporozoites. Once *P. falciparum* and *P. vivax* infection is established, we will use RNA-Sequencing to generate transcriptomic profiles of each infection stage. This molecular analysis will identify key genetic markers during liver development, especially for the elusive *P. vivax* hypnozoite, a persistent liver stage that is linked to malaria relapse, allowing more targeted drugs and vaccines to be developed.

The Role of Purinergic Signaling in Human Cytomegalovirus Infection

Saisai Chen
Maciej T. Nogalski
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Human cytomegalovirus (HCMV) is a β-herpesvirus that infects a large fraction of the adult population worldwide. HCMV infection can cause central nervous system damage in neonates, complications in transplant recipients, and chronic diseases in immunocompromised individuals. HCMV has also been associated with the etiology of several human cancers. Such broad clinical implications stem from the ability of the virus to modulate multiple host cell processes. One mechanism by which this can occur is through receptor-mediated signaling. Specifically, the current study aims to determine the effects of purinergic signaling on HCMV infection. Purinergic receptors are cell-surface receptors that are activated by extracellular nucleotides and nucleosides. Their activation triggers signaling cascades that lead to the regulation of various cell processes, including cell proliferation, differentiation, and migration. Our data from RNA sequencing show that HCMV infection in human fibroblasts alters the expression of several components of the purinergic signaling pathway, but the biological implications of this phenomenon have yet to be examined. To explore the significance of purinergic signaling during HCMV infection, we treated infected cells with antagonistic pharmacological agents that target various components involved in the purinergic signaling system. Differences in viral protein expression and progeny production between treated and untreated cells were observed. Conversely, the efficiency of viral entry was not impacted by the treatments. Of the six compounds under study, kaempferol, a potent antagonist of the $P2Y_2$ purinergic receptor resulted in the most pronounced effects on the progression of viral infection. Interestingly, the $P2Y_2$ receptor was found to be the most upregulated of the purinergic receptors in HCMV-infected cells. Although preliminary, our results suggest that the purinergic signaling pathway may be a potential target for antiviral therapy. However, further research should elucidate the molecular mechanisms by which purinergic signaling affects HCMV replication and progeny production.

The Role and Regulation of Elf5 Methylation in Breast Cancer

Abrar Choudhury
Rumela Chakrabarti
Adviser: Yibin Kang

E74-like factor 5 (Elf5) plays a crucial role in mammary gland development as a master regulator of cell fate determination. It inhibits mammary stem cells in the basal layer and turns luminal progenitors into differentiated cells. Our lab has shown that Elf5 is downregulated in breast cancer tumors, when compared to normal breast in breast cancer data sets. Furthermore, previous experiments have found that Elf5 inhibits the epithelial-mesenchymal transition and prevents metastasis by repressing Snail2. Other studies have shown that Tet and Dnmt family proteins regulate Elf5 through demethylation and methylation respectively in the context of normal mammary gland development.

Here, we study the role and regulation of Elf5 through methylation in the context of breast cancer. We explore the functional relevance of Elf5 methylation by injecting breast cancer cells into the mammary fat pad and lateral tail veins of mice to test tumorigenesis and metastasis respectively. We show that treating 4T1 breast cancer cells with 5-azacytidine (Aza), a demethylating agent, decreases both tumor formation and metastasis, phenocopying Elf5 overexpression. Furthermore, blocking Elf5 expression in Aza-treated cells using short hairpin RNAs rescues the wild type 4T1 phenotype, confirming that the phenotypes are due in part to changes in Elf5 expression.

Using qPCR, we also show that Tet and Dnmt expression correlate and inversely correlate with Elf5 expression respectively at different stages of the C3 mouse breast cancer tumor model. These results suggest that Tets and Dnmts play a crucial role in breast cancer tumor progression through Elf5 regulation. Further studies will include testing the functional role of overexpressing and knocking down Tets and Dnmts in cancer cells to better understand how they regulate Elf5.

Recognition and Assembly of Mutant Outer Membrane Protein A by the β-Barrel Assembly Machine in Escherichia coli

Lillian Dattilo
Adviser: Thomas Silhavy

The outer membrane (OM) of Gram-negative bacteria is a lipid bilayer containing β-barrel proteins. Assembly of outer membrane proteins (OMPs) is essential in Gramnegative bacteria such as Escherichia coli and is catalyzed by the β-barrel assembly machine (Bam). How Bam recognizes and folds nascent OMP substrates is poorly understood. The essential Bam components BamA and BamD are known to interact with substrates independently. A conserved signature sequence, termed the β-signal, has been identified in the C-termini of many OMPs and is hypothesized to target OMPs to Bam. This sequence is necessary for binding of some OMPs to BamA and BamD in vitro, but its in vivo role in recognition of OMPs by Bam remains unclear. Four mutations to the proposed β-signal of OmpA, a model OMP, were shown to reduce levels of mature OmpA. One of these mutations, ompAG186R, caused significant impairment to OM biogenesis, resulting in dominant drug permeability defects. Synthetic lethality with ΔbamB indicates ompAG186R disrupts normal Bam interactions. Induction of the σE response and the essentiality of the protease DegP in the mutant strain suggest misfolding and impaired assembly of mutant protein. However, this is contradicted by phage sensitivity assays, which demonstrate assembly of OmpAG186R, and growth and heat modifiability assays, which indicate proper Bam function. Formation of large pore OmpA in the mutant strain best explains these data. OmpA can adopt two different β-barrel conformations, a small and large pore, but the large pore form has yet to be isolated or characterized in vivo. This study may therefore be useful in shedding light on not only the stepwise process of OMP biogenesis but also the in vivo properties of large pore OmpA.

Mapping cerebello-thalamic and cerebello-ventral tegmental neural pathways using transsynaptic viral tracing

Shruthi Deivasigamani
Thomas J. Pisano
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The cerebellum, classically involved with motor coordination, is known to act as a perceptron to correct motor errors and refine movement. However, recent fMRI and tracing studies have suggested a nonmotor, cognitive role for the cerebellum as well: using a cerebello-midbrain-neocortical synaptic pathway, it has been proposed that the brain refines social learning and reward processing using a similar error-correction model. In order to understand the nature of these pathways, the research outlined here seeks to investigate the neuronal connectivity and topography of these circuits using viral transsynaptic tracing—a relatively novel form of neuroanatomical tracing that allows us to map cerebellar outputs beyond the deep cerebellar nuclei. Midbrain regions under investigation include the thalamus and the ventral tegmental area (VTA), both of which have been implicated in reward processing. The first aim will determine cerebello-thalamic connections from cerebellar lobule VI-a and VI-b in model mice using herpes simplex virus I H129 strains. Varied expression across animals with different injection sites will allow us to create a projection map using a generalized linear model. Preliminary experimentation has established cerebello-thalamic connections, and has also suggested differential topography from midline to lateral injection points in lobule VI-a. Using an extensive time series, the viral rate-of-spread for fluorophore-tagged H129 was established as approximately one synapse every 24 hours and the ideal incubation period for anterograde cerebellar injections as approximately 88 hours. The second aim will determine cerebello-VTA projections using retrograde-acting pseudorabies virus (PRV); PRV-Brainbow in conjunction with DAT-Cre transgenic mice will also allow for classification of these pathways as dopaminergic or non-dopaminergic. Understanding these two cerebello-midbrain-cortical pathways will establish neural topography and shed light on pathway disturbances that could result in disrupted reward processing.

Probing Erk activity in time and space

Dan DiGiorno
Adviser: Jared Toettcher

The Ras/Erk cascade is crucial in many stages of eukaryotic cell life, especially during development, and is highly conserved throughout species. Direct optogenetic stimulation of this pathway has made it possible to disentangle this system from the many interconnected pathways cells use to govern decisions. Using this tool, it is possible to spatiotemporally pattern light inputs to test signal transmission. Previous studies have identified mutations to the D recruitment site (DRS) and F recruitment site (FRS) through which Erk binds and interacts with its upstream and downstream partners. Here, we characterize the signal transmission of five Erk mutations. For both binding sites, the mutations produced a range of phenotypes such as varied amplitude, permanent activation, and inactivation. This highlights the complex nature of these binding sites and wide range of binding partners. More work will need to be done to determine the mechanism by which these phenotypes are produced. Future work will focus on cloning the optogenetic construct, Erk reporter, and a nuclear tag onto one plasmid to allow for even expression. This will provide a powerful tool to also probe other mutations in the Ras/Erk pathways, such as Ras and Raf, which have been implicated in a number of cancers. In addition to our work on the temporal stimulation of these mutants, we also aim to study how spatial and temporal features of Ras/Erk specify early development. For this end, we have generated a line of *Drosophila melanogaster* containing our optogenetic system in order to test signaling patterns and determine which factors actually contribute to cell fate.

Modulating Neural Stimuli to Examine *Drosophila melanogaster* Male Courtship Song Patterning

Tina Doan

Adviser: Mala Murthy

Drosophila melanogaster, more commonly known as the fruit fly, produce an acoustic signal during the courtship ritual. This signal comes from male flies extending and vibrating one wing with the hopes that the resulting song attracts the attention of females who want to copulate. The courtship song consists of 2 vibration modes, sine and pulse, which are organized into bouts. Males also dynamically modulate their songs using sensory stimuli. This modulation occurs on the timescale of milliseconds, facilitating the study of the neural activity underlying these behavioral changes. The *Drosophila* song circuit has been partially mapped, and although 4 neurons- P1, pIP10, dPR1, vPR6- are known to be involved, their specific roles, relationships, and effect on song structure are still unknown. This project aims to elucidate these neurons' roles by using optogenetic tools to non-invasively activate P1, pIP10, and vPR6 and examine the song properties modulated by different stimulus parameters, such as frequency and intensity. Singing was induced in young, single-housed male flies using red activatable channelrhodopsin (ReaChR) and a fly-on-the-ball setup. For these 3 neurons, it was found that the probability of song production increased with intensity, especially those greater than 3 V. For higher intensities, the probability also increased with frequency. Future work will examine the effect of stimulus parameters on inter-pulse interval (IPI) and bout length. The stimuli will also be modulated to determine the minimum unit of song that can be elicited.

Genetic Testing in the Era of Precision Medicine

Swetha Krishna Doppalapudi

Adviser: Shirley Tilghman

The Precision Medicine Initiative of 2015 promises to deliver insights on the genetic underpinnings of various complex diseases by investigating genetic and health data from over one million individuals in the United States. At the current rate of technological progress in the field of genomics, it is almost inevitable that large scale sequencing techniques become commonplace in the field of medicine. Although genotyping technologies are improving quickly, the medical establishment is still struggling with the clinical validity and utility of the vast amount of information gained from genetic testing. Some genetic variants have straightforward association with disease-risk, but most do not, and thus genetic tests pose a unique risk to patients by providing them with potentially unreliable interpretations of their genomic information. The Food and Drug Administration (FDA) is currently attempting to regulate genetic tests through their proposed framework for Laboratory Developed Tests (LDTs). After a careful consideration of the FDA's proposed framework, I recommend that Clinical Laboratory Improvements Amendments are strengthened, a risk-based clinical utility assessment system is adopted, and the "off-label" use of LDTs is allowed. These regulatory steps should protect consumers from faulty tests, while also allowing for genomics to be usefully integrated into the medical infrastructure.

Mechanisms of Neural Synchrony:
Developing a Novel Behavioral Paradigm Featuring Cross-Modal Attention

Kristen Duncan

Adviser: Timothy Buschman

Cognitive control encompasses a range of cognitive processes required for selecting behaviors critical to attaining a particular goal. Attention is a subset of cognitive control, and acts to select for what the brain's limited processing capacity is used. It has been suggested that the synchronization of oscillations within and between areas is the mechanism underlying attentional processes; however, a causal link between the two has yet to be found. As synchronous patterns are disrupted in many neuropsychiatric and neurodevelopmental disorders, including schizophrenia, autism spectrum disorders, Parkinson's, and Alzheimer's diseases, we are motivated to understand the mechanisms underlying synchronous activity. The ultimate goal of this research is to examine the possibility of a causal link between attention and gamma-band oscillations; therefore, it is first necessary to develop a paradigm that tests a mouse's ability to attend. In this case, we are using cross-modal attention, requiring the animal to attend into and out of the somatosensory and auditory modalities. To do this, we trained two cohorts of mice on two behavioral tasks: the first, with the goal of teaching subjects to attend into the auditory modality using a sound stimulus, and the second, with the goal of attending to into the vibrissae modality using a whisker stimulus. As only one subject from the first cohort learned to attend to the sound stimulus, and four subjects learned to attend to the whisker stimulus, we have identified several ways to improve upon future paradigms.

Effect of race and smoking status on frequency of oncogenic driver alterations in non-small cell lung cancer patients at Abramson Cancer Center

Elshaddai Ephrem

Adviser: Virginia A. Zakian

Non-small cell lung cancer (NSCLC) is an exemplar of “personalized medicine” in oncology, a term defined by development of genotype-directed targeted therapies against activation of oncogenic driver signaling. Treatment of advanced stage NSCLC involves tumor genotyping to identify oncogenic driver alterations (ALK rearrangement, EGFR mutations, and KRAS mutations) that predict clinical response to targeted therapies. The prevalence of driver alterations in minorities is understudied, but differences in tumor biology are hypothesized to underlie racial disparities in lung cancer outcomes. I sought to determine the association between race and mutational positivity in NSCLC. I conducted a retrospective study of the frequency of ALK rearrangement, EGFR mutation, and KRAS mutation among patients with NSCLC at the ACC, stratified by demographic and clinical variables. Among 611 patients whose tumor had at least ALK FISH testing, the overall frequency of ALK rearrangement was 4.6%; of EGFR mutation 11.4%; and of KRAS mutations 28.1%. The findings of this study indicate that race is not significantly associated with the frequency of driver alterations in NSCLC. Race should not be considered when deciding which patients to screen for driver alterations. Smoking status was an independent predictor of harboring an oncogenic driver alteration in tumor. ALK-positive NSCLC and EGFR-positive NSCLC were more common in non-smokers, and KRAS-positive NSCLC was more common in smokers. Differences in pattern of oncogenic driver alterations between smokers and non-smokers supports theory that lung cancer in non-smokers is a distinct disease from that in smokers and may lead to elucidation of the molecular pathways that lead to lung adenocarcinoma.

Introduction of Fluorescent Reporters into *Oxytricha trifallax*

Ysa Esquilin

Adviser: Laura Landweber

The freshwater ciliate *Oxytricha trifallax* possesses an unusual genome architecture that makes it well-suited to the study of chromatin structure, telomeres, gene splicing, and DNA repair. Like all ciliates, *Oxytricha* has a germline nucleus that undergoes large-scale DNA deletion and rearrangement to yield a mature somatic nucleus containing tens of thousands of small, gene-sized chromosomes. Current methods for introducing changes into the *Oxytricha* genome are limited in their ability to introduce non-native genes. Using overlap extension PCR, the sequences for red fluorescent protein (mRuby2) and the fluorescent RNA aptamer Mango were inserted into the chromosomes coding for Otiwi1, Alba and Chitin Recognition Protein (CRP). The resulting constructs were then verified by TOPO cloning and sequencing. Future work will involve transformation of *Oxytricha* cells with each construct and verification of expression by fixed and live-cell fluorescent imaging and by RT-PCR. Successful transformation of *Oxytricha* could facilitate the use of currently-unavailable methodologies in this model organism such as FACS, time-lapse microscopy, and tagging of proteins/RNAs for immunoprecipitation.

Characterizing the Role of Genetically Defined Central Brain Neuronal Clusters in the Processing of Courtship Song in *Drosophila melanogaster*

Ramie Fathy
Adviser: Mala Murthy

Across species, decisions are driven by internal states, personal experience, and sensory input. Acoustic communication between animals represents one medium through which one animal can influence another's behavior. This relationship can be studied through the courtship song of *Drosophila melanogaster* and its effect on female receptivity to courtship. Previous work has implicated the pC1 and pCd neuronal clusters in the *Drosophila* protocerebrum in processing auditory and pheromonal input during courtship and mediating their effects on female receptivity, implicating them as critical decision making neurons linking song processing to female behavior. To test this possibility, we use an assay that enables us to quantify the behavioral response of the female to male song by simultaneously recording male song and tracking the flies' positions. Our previous work has found that silencing of the female's pCd and pC1 neurons results in significantly decreased times to copulation, supporting our hypothesis that the pCd and pC1 neurons encode positive aspects of courtship song and promote female receptivity. To test if the pCd and pC1 neurons have a prolonged effect on female behavior during courtship, particularly on her behavioral response to song, we have modified our courtship assay to allow us to optogenetically activate either the pCd or pC1 neurons in a female prior to courtship and observe the effects on the female's time to copulation. While we require more data before making conclusions about the pCd neurons, preliminary analysis suggests that activation of the pC1 neurons results in significantly shorter times to copulation and greater overall copulation compared to wildtype flies. Ultimately, we hope to use our findings to design a closed-loop feedback system that enables us to modulate courtship song playback based on the female's behavior or the response of the neurons, or conversely, to perturb the activity of the neurons based on sensory input. Our work will provide insight into how the brain represents auditory information and integrates it with other sensory inputs in order to mediate its effects on decision-making and behavioral output.

Investigating the dual function of the *Drosophila* hnRNP Glorund: Splicing factor and translational repressor

Amelia Foss
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Heterogeneous nuclear ribonucleoproteins (hnRNPs) are necessary for the proper spatio-temporal control of gene expression during development. Glorund (Glo) has been identified as the sole *Drosophila* homolog of the mammalian F and H families of hnRNPs, which are known to regulate splicing through G-tract binding motifs. In *D. melanogaster*, Glo is a member of a protein complex that alternatively splices *ovarian tumor (otu)* during early oogenesis, though the mechanism remains unknown. G-tracts present in *otu* mRNA suggest that Glo may bind *otu* directly to regulate splicing. However, Glo has also been identified as a translational repressor of *nanos (nos)* via the *nanos* TCE during oogenesis, making it the first F/H hnRNP family member or homolog implicated in translational repression. Interestingly, mutations in Glo's TCE-binding motifs result in an adolescent phenotype characteristic of a lack of TCE-mediated repression in the CNS and the disruption of the hormone pathway regulating ecdysis. This suggests Glo may target *nos*-TCE-like mRNA structures for translational repression in the CNS, a hitherto unknown function of Glo. This project investigates the dual functions of Glo as both a splicing factor and translational repressor. We expect to find that Glo directly binds the G-tracts of *otu* to regulate alternative splicing during oogenesis, and that Glo is active as a translational repressor in the CNS, moderating the ecdysis hormone pathway through TCE-binding motifs.

PROMETHEUS

Examining learning and memory in the mouse primary visual cortex

Alistair M. Glidden
Jan Homann
Adviser: Michael Berry

Intelligence is not just the ability to perceive stimuli and make decisions – it also requires the capability to foresee future events that fall into a pattern. Intelligent behaviors such as learning and memory must therefore require animals to make predictions about their environment and change their behaviors based on spatial or temporal cues. We test this hypothesis by studying the mouse primary visual cortex (V1) while the animal views repeating temporal patterns of visual stimuli. In the first phase, mice (N = 5) were shown a pattern of repeated abstract stimuli, either gratings or images with many small line segments (Gabor functions), which were formed into sequences of length 2 to 7 frames. At random intervals, a novel frame that violated the ongoing pattern was displayed; the mouse had to lick immediately thereafter to receive a water reward. Our results clearly show that the mice learned to recognize the violation frame and restrict their licking to the reward window. Interestingly, the mice fell into two learning categories: those who showed a higher but uniform baseline rate of licking; and those who initially licked at every appearance of the pattern, but showed rapid learning and superior performance once the frequency of violation frames was decreased. In contrast, decreasing the frequency of violation frames worsened the performance of the mice in the first category. Based on these results, we will next present naïve mice with temporal sequences and use two-photon Ca^{++} fluorescence imaging to measure the GCaMP6f signals from ~200 neurons in layer 2/3 of V1. We have preliminary data suggesting that cells can be categorized as showing a sustained stimulus response, a transient stimulus response, or no stimulus response. As the mouse is repeatedly presented with a sequence, the transient responses to violation frames increase, and the sustained responses exhibit shortened reaction times, both of which suggest learned patterns. The final phase of the study will then combine imaging of V1 with behavioral training, and track the three cell categories as the mouse learns the stimulus pattern. If the transient violation response and anticipatory time-shift increase with the mouse's accuracy, we will have evidence that prediction underpins learning, memory, and, ultimately, intelligence.

Characterization of Chikungunya Virus: Emergence and Responses to Outbreaks

Yendé A.J. Grell
Adviser: Adel Mahmoud

Chikungunya (CHIKV) is a re-emerging mosquito-borne alphavirus of the Togaviridae family that causes acute febrile arthralgia. The virus contains a 12kb singlestranded, positive sense RNA genome, circulates in zoonotic and urban cycles, and is transmitted by mosquitos of the Aedes species. Over the past ten years, a convergence of factors including a mutation in the E1 envelope glycoprotein have facilitated the geographic expansion of CHIKV infection to more than fifty countries, affecting over 2 million persons in the Indian Ocean, Pacific Ocean, and Caribbean regions. This thesis examines current knowledge on CHIKV including its phylogeny, structure, and pathogenesis. It has been found that CHIKV encodes four nonstructural proteins that participate in the replication cycle of the virus as the replicase complex and capsid and envelope structural glycoproteins with antigenic properties. Examination of the Alanine to Valine mutation in the IOL CHIKV strain also offered further insight into the emergence of this virus via Ae. albopictus transmission. This work considers the potential for various control strategies against CHIKV in light of these known viral properties. Promising antiviral candidates include broad spectrum drugs, or those previously licensed against other viruses, and vaccine candidates reflect modern vaccine technologies for delivery of the defined CHIKV antigens. In the absence of CHIKV treatment and prophylaxis, novel species-specific approaches to vector control that utilize bacterial endosymbionts to block pathogen transmission and DNA technology to genetically modify mosquitos may rapidly curtail the spread of not only CHIKV, but other mosquitoborne infections that are constantly emerging or re-emerging as global pathogens.

Quantification of Bicoid Protein in *Drosophila melanogaster* embryos during Early Development

Ahmed Hassan

Adviser: Eric F Wieschaus

Morphogenic gradients regulate developmental progress in the early *Drosophila melanogaster* embryo. The gradient of the maternally imputed transcription factor, Bicoid, extends across the anterior-posterior axis and is responsible for head and thorax development. Bicoid is known to bind several hundred sites in the genome and is prevalent during the syncytial blastoderm stage of *Drosophila* development. Previous studies quantifying Bicoid protein concentration have provided molar estimates at mid-embryo length ranging from 8 – 200 nM of Bicoid present in nuclei during the fourteenth nuclear cycle occurring at the end of the syncytial blastoderm stage. Determining the number of Bicoid molecules in nuclei is further complicated by lack of evidence regarding the percentages of Bicoid present in the yolk, cytoplasm and nuclei which dominate embryo space during the syncytial blastoderm stage. We set out to obtain a true molar value of Bicoid protein in the developing embryo by confirming molarity results across quantitative Western blotting, fluorescence assays, ELISA and microscopy. As a standard, we made use of rGFP protein and mutant embryos expressing Bicoid equally across the anterior-posterior axis at known expression levels based on fluorescence intensity relative to wild type expression. In establishing the molarities across the anterior-posterior axis and across the lifetime of the gradient, we will be able to confirm a model of Bicoid gradient formation and determine the nuclear concentration across the gradient. The Bicoid gradient can be modeled via an exponential decay gradient based on known fluorescence intensity levels corresponding to Bicoid expression. Preliminary results from quantitative western blotting and fluorescence assays have indicated a total of ~1.5×10^8 molecules of Bicoid protein in one embryo during the syncytial blastoderm stages corresponding to a nuclear Bicoid molarity of ~90 nM. This further supports existing unpublished data from studies on Bicoid concentrations necessary for in vivo-target gene binding.

Mapping of Projections from Superior Colliculus to Frontal Orienting Field through Mediodorsal Thalamus in Rat

Nicholas Horbelt

Adviser: Carlos Brody

Decision making is a complex process of both the gathering of pertinent sensory stimuli (termed accumulation of evidence) and the analysis of gathered stimuli to result in a choice, which undoubtedly involves multiple brain areas. Behavioral tasks in which mammals use auditory, visual or somatosensory data to make a binary choice are a common method to measure and perturb decision making. Studies in which areas of the brain are either inhibited or measured for activity during such tasks have resulted in the identification of several candidate areas thought to be involved in decision making, including the posterior parietal cortex (PPC), frontal orienting field (FOF; frontal eye field (FEF) in primates), and superior colliculus (SC). Furthermore, studies conducted on rats using an auditory decision making task, called the Poisson click task, have determined that inhibition of the FOF does not significantly affect the accumulation of evidence step, but rather the choice step in decision making, indicating a higher order role in the decision making process. Inhibition of the SC results in a similar bias to FOF inhibition during decision making tasks of an auditory or visual nature. Thus, it is pertinent to determine how these areas are connected in the brain to determine the nature of the decision making circuit. While the connectivity between some of these areas – such as reciprocal connections between PPC and FOF, and a monosynaptic connection from FOF to SC – has been elucidated, much of this information resulted from primate studies. Here, we will study the projections from the SC to the FOF in rat, which has been shown to be mediated through the mediodorsal (MD) nucleus of the thalamus in primates. Further behavioral studies in which neurons in this ascending pathway will be inactivated during the Poisson click task will be conducted.

Preliminary Analysis of R-SNARE binding to SM protein Sly1

Sarah Howells

Adviser: Fred Hughson

Cell trafficking is a process by which cargo-carrying vesicles fuse with target membranes within the cell to release proteins into specific cellular compartments. An R-SNARE protein on the vesicle membrane zippers together with Q-SNARE proteins on the target membrane to reduce energetic barriers to membrane fusion. Sly1 acts in vesicle fusion in both anterograde and retrograde trafficking pathways from the endoplasmic reticulum (ER) to the Golgi apparatus by binding Q-SNAREs on the target membrane. However, the possibility of functionally important interactions between Sly1 and R-SNAREs has yet to be explored. Recent work in the Hughson lab has demonstrated that the SM protein Vps33 binds the R-SNARE Nyv1 in a surface groove homologous to that covered by a "lid" formed by a short helical region, α20, on Sly1. Mutations that destabilize this short helical region of Sly1 act as dominant suppressors of lethal mutations of multiple essential trafficking proteins. Here, we aim to determine whether binding between Sly1 and its cognate R-SNARE proteins occurs at the surface groove underneath the lid formed by the α20 region of Sly1. Sec22 and Ykt6 were purified as His tagged MBP fusion proteins, whereas Sly1 and Sly1 α20Δ proteins were purified as GST tagged proteins. The two R-SNAREs Sec22 and Ykt6 were tested in pull-down assay with wild type Sly1 protein and Sly1 α20Δ proteins. Characterizing whether such a binding interaction between Sly1 and R-SNAREs exists would determine if multiple SM family proteins facilitate and regulate R-SNARE entry into the SNARE complex.

***Flavobacteria*: A new treasure trove of bioactive compounds**

Phoebe Huang

Adviser: Mohamed S. Donia

The emergence and spread of multidrug-resistant bacterial pathogens has made the development of novel antibiotics an important global health concern, especially as many drugs previously used to treat bacterial infections are becoming ineffective. Bacteria are rich resources of bioactive small molecules, encoded by biosynthetic gene clusters (BGCs), that can be developed and used as antibiotics. Actinobacteria have traditionally been thought to be the best producers of these small molecule drugs. However, in this study, we used bioinformatics to predict BGCs in *Flavobacteria* strains with similar structures and properties to a previously identified antibiotic-encoding gene cluster, and found that 9 different species have big BGCs in their genomes that potentially encode bioactive compounds**.** Next, we performed chemical extraction of the bacterial supernatants and cells for antibacterial and antifungal assays. We employed the disc diffusion assay and used four indicator strains: *Staphylococcus aureus, Escherichia coli, Micrococcus luteus,* and *Candida albicans* that represent a spectrum of Gram-negative and Gram-positive bacteria and fungal pathogens to test the activities of these chemical extracts. The results showed various degrees of antibacterial and antifungal activity in the crude extracts, and one extract from *Aquimarina muelleri* showed strong inhibitory activities against all four indicator strains. In the future, we plan to identify the active components of these extracts using mass spectrometry and NMR. In addition, we also initiated the development of tools to genetically manipulate these *Flavobacteria* strains for using them as heterologous expression hosts. We were able to introduce a foreign plasmid into one of the nine strains via bacterial conjugation. Our findings demonstrate the great potential of *Flavobacteria* strains for producing bioactive compounds, which warrants further investigation.

Cell cycle control of planar cell polarity protein Celsr1 during late phases of mitosis

Colby Hyland
Rezma Shrestha
Adviser: Danelle Devenport

The maintenance of planar cell polarity (PCP), or the coordinated alignment of polarized components along the epithelial plane, requires specific mechanisms during mitosis when cell structure drastically changes. One mechanism that works to maintain polarity during mitosis is the bulk internalization of cell surface PCP components into endosomes, after which equal redistribution and subsequent polarization occurs in daughter cells. We showed previously that the internalization of the core PCP component Celsr1 is tightly coordinated with the onset of mitosis by the cell cycle regulator Plk1. Phosphorylation of the Celsr1 C-terminal endocytic motif by Plk1 is required for the internalization of Celsr1 into clathrin-coated endosomes. However, the mechanism whereby Celsr1 is retained and then recycled back to the membrane in concert with cell cycle exit is not known. We show here that the premature inactivation of Cdk1 results in the return of Celsr1-containing endosomes to the membrane. We also show that Plk1 inactivation during metaphase arrest causes premature return of Celsr1-containing endosomes to the membrane. These findings suggest that Plk1 kinase activity is essential for the retention of Celsr1-containing endosomes during mitotic progression and that Cdk1 inactivation may be the upstream cell cycle signal that triggers Celsr1 recycling to the membrane in late phases of mitosis. Future work will involve elucidating the mechanism whereby cell cycle regulators directly or indirectly control Plk1 activity at Celsr1 endosomes and the time-specific recycling of Celsr1 to the membrane. The present study will characterize further how planar cell polarity is consistently maintained across cell generations.

Examining the Effects of Pseudorabies Virus (PRV) Infection on Neuropeptide-Y (NPY) Transport in Primary Neuronal Axons

Jolie Jean
Ian Hogue
Adviser: Lynn W. Enquist

Pseudorabies virus (PRV) is a pathogenic alpha herpesvirus that invades the peripheral nervous system (PNS) of its host. Spread of alpha herpesviruses from the PNS to the central nervous system can cause a rare but deadly encephalitis, highlighting the medical importance of understanding the mechanisms of alpha herpesvirus transport. Nevertheless, the effect of PRV transport on the dynamics of physiological cargo transport is poorly understood. Recent studies have determined that the microtubule-dependent kinesin, KIF1A, is necessary for axonal sorting and anterograde transport of PRV virions. KIF1A has also been well studied for its role in anterograde transport of neuropeptide Y (NPY). Efficient transport and release of NPY is important for physiological functioning of both the nervous and immune systems. Since both PRV and NPY utilize KIF1A for anterograde transport, we are interested in determining whether PRV infection influences the dynamics of vesicles containing NPY. Using fluorescently labeled PRV capsids as well as fluorescently labeled NPY, we hypothesize that NPY transport will be decreased upon PRV infection. Because both PRV virions and NPY utilize KIF1A, our current model therefore posits a competition for KIF1A between PRV and NPY, leading to an increase of PRV transport and a decrease in NPY transport over time. Thus far, we have found that the adenoviral vectors efficiently deliver NPY-EGFP, with the signal localized to the cytoplasm, axoplasm and the *trans*-Golgi network. Using these reagents, we validated the dynamics of PRV capsids in axons as well as the dynamics of NPY in axons, which were both seen to be consistent with previously published data. Future directions focus on the utilization of both these reagents simultaneously to test the hypothesis that PRV infection affects NPY transport dynamics. In doing these experiments, we hope to elucidate the molecular underpinnings of anterograde transport of physiological cargo in both healthy and infected neurons, which will contribute to the understanding of the effects this important pathogen has on neuronal function.

Characterization of Benenodin-2

Caroline Kim
Adviser: A. James Link

Lasso peptides comprise a class of ribosomally-synthesized and post-translationally modified natural products characterized by their slipknot-like structure. This structure consists of a ring formed by an isopeptide bonded N-terminus with a tail that threads through the ring. The tail is kept in place by disulfide bonds or bulky side chains called “steric locks” on both sides of the ring. Their highly constrained topology allows for great stability and resistance to proteolytic degradation, making them ideal for many peptide therapeutics. The benenodin family of lasso peptides was identified through genome mining studies of putative lasso peptide gene clusters. Benenodins contain four gene clusters: abeA, abeB, abeC, and abeE. The maturation enzymes, abeB and abeC, cleave and cyclize the mature lasso peptide, abeA. The abeE gene cluster encodes an isopeptidase that linearizes the peptide. The benenodins are very closely related to the previously characterized astexin family of lasso peptides. However, unlike the astexins, the benenodins have been shown to be very thermostable with very little unthreading even after several hours of heating. Thus, we sought to examine potential elements in benenodin-2 that allowed it to be so thermostabile. We removed each of benenodin-2’s hypothesized steric locks to determine if its thermostability was provided solely by its steric locks. Results showed little unthreading after twelve hours of heating suggesting that another mechanism works in conjunction with benenodin-2’s steric locks in order to confer stability to the peptide.

Investigating motile cilia defects as causal genetic factors for scoliosis

Eunice Y. Lee
Adviser: Rebecca Burdine

Scoliosis is a three-dimensional musculoskeletal deformity that is characterized by spinal curvatures of 10 degrees or more. It is largely classified into congenital scoliosis, which results from vertebral malformations present at birth, and idiopathic scoliosis, which often develops during adolescence. While mutations in genes encoding fibrillar collagens and Wnt/β-catenin signaling components have been associated with congenital scoliosis, much about idiopathic scoliosis remains unknown due to phenotypic and genetic heterogeneity. Cilia are microtubule-based protrusions found on the surface of nearly all cell types in the human body. Motile cilia, such as those found on epithelial cells that line the respiratory tract, generate fluid flow in an ATP-dependent manner. Here, we describe a connection between cilia motility and a scoliosis-like condition in zebrafish.

Previous unpublished studies of zebrafish mutants *zoolander* (*zoo*), *dyx1c1* and *c21orf59* have shown that in addition to an early curved body axis, laterality defects, and abnormal otolith numbers that are characteristic of motile cilia mutants, mutants develop spinal curvatures as juveniles that progress into severe scoliosis-like curves. Thus, we propose that motile cilia defects may be causal in scoliosis. Motile cilia mutants exhibited no notochord or congenital vertebral malformations, and developed scoliosis as juveniles, which suggests that motile cilia mutants present idiopathic, not congenital, scoliosis.

In the future, we will analyze the biological mechanisms that underlie spinal curvature development in mutants with defective motile cilia. This will be accomplished by probing for cellular discrepancies in the vertebral column between wild-type and mutant scoliotic fish. We will be particularly interested in differences in gross cell morphology, cell proliferation and apoptosis, and planar cell polarity, as these are all candidates for causative curvature formation downstream of cilia motility. Our primary hypothesis is that motile ependymal cilia within the spinal column are required to maintain a straight backbone throughout life.

Visualizing the Structures of Celsr1-containing Endosomes During Mitosis

Yun Liang
Bryan Heck
Adviser: Danelle Devenport

Planar Cell Polarity (PCP) coordinates the alignment and direction of cellular structures and behaviors across tissues. This uniformed alignment can be exemplified by the anterior to posterior orientation of the hair follicles in mammalian skin. However, rapidly proliferating cells, such as those found in the skin, pose a great danger for the maintenance of PCP as PCP proteins might be mis-localized in the resulting daughter cells. Previous studies have demonstrated that these cells address this risk by internalizing their transmembrane PCP proteins through endocytosis during mitosis, with Celsr1 playing a critical role. However, the precise mechanism of how this endocytosis occurs or how proper PCP alignment is re-established in the region after mitosis remains unclear. Preliminary data suggest that the mechanism of internalization of transmembrane PCP proteins involves a process termed “trans-endocytosis”, whereby PCP complexes interacting between adjacent cells are co-internalized into the dividing cell. In this study, we aim to elucidate the topology of PCP vesicles and the structural changes that occur during and after trans-endocytosis through electron and fluorescent microscopy as well as to identify which domain(s) of Celsr1 are required for the initiation of trans-endocytosis. We suspect that trans-endocytosis is carried out through the formation of a double membrane endosome that is then either maintained or resolved to a single membrane endosome at the end of mitosis prior to fusing with the cell membranes of the daughter cell to restore the local PCP alignment. Additionally, we believe that the intercellular Celsr1-Celsr1 homotypic interaction between adjacent cells is crucial for the formation of the predicted double membrane endosome. These findings could offer insight into the specifics of a novel way in which cells share their contents.

Analysis of Histones at the Midblastula Transition Across Various Species of *Drosophila*

Jack Mazzulo
Adviser: Amanda Amodeo

Drosophila embryos begin development with incredibly fast and synchronous divisions, primarily regulated by maternally deposits. When these factors diminish and *de novo* transcription activates, the embryo is said to be going through the maternal to zygotic transition (MZT). During the MZT, however, there is a clear point, called the midblastula transition (MBT), where bulk zygotic transcription is initiated. One proposed theory for the timing of the MBT is that there exist limiting maternal factors that have a direct effect on the timing of the MBT, and that as the nuclear to cytoplasmic ratio increases with each cell division, these limiting factors are exhausted, inducing the MBT. Research by Amodeo, et al. showed in *Xenopus* that these factors might be histones. Expanding these findings, we wanted to see to what extent the nuclear density and histone levels of embryos at the MBT varied across species, and if these two components covaried. We obtained 24 different species of *Drosophila*. By collecting and lysing embryos at MBT, we ran fluorescent westerns using fluorofores Alexa 488 and Alexa 647 to stain for histones and tubulin (our loading control). After imaging the western on a Bio-rad Chemidoc, we quantified the levels of histones for four of the species, and normalized them to total protein within species, and then to *D. melanogaster* across species. Future steps include measuring the amount of nuclear material at MBT for each species by fixing embryos with formaldehyde and staining them with Hoescht and phalloidin. After imaging them with a confocal microscope, we can count the number of nuclei in each. Finally we will plot the histone levels and nuclear density for each species and determine to what extent they vary and covary. Using these plots, we hope to find support for histones as titratable factors within *Drosophila*.

Meta-analysis of breast carcinoma transcriptomes demonstrates correlation between differential splicing of RAC GTPases and tumor aggressiveness

John H D McNeil

Adviser: Alexei Korennykh

Cancer, one of the leading causes of death worldwide can be broadly categorized into two types: Sarcoma and Carcinoma. Carcinoma, a cancer originating in epithelial tissues, makes up most of the most common forms of cancer, such as breast, lung, and prostate1. While recent advances in surgical and chemotherapeutic techniques have increased the survival rate for localized cancer, the 5-year prognosis for metastatic cases has not improved nearly as much2. To this end, research has been done to identify early indicators of metastatic potential, as well as to elucidate the method of metastasis. Carcinomas can only present an invasive phenotype by initiating Epithelial to Mesenchyme Transition (EMT), a process of cell differentiation that occurs naturally during development and wound healing, which downregulates tight cell junctions and upregulates cell motility. Previous work has suggested that there is a correlation between EMT potential in breast cancers and a change in splicing patterns of the gene RAC1, a signaling GTPase with effects on the cytoskeleton3. Collecting multiple RNAseq datasets of breast carcinoma cells, this paper examines whether this differential splicing pattern holds true for other members of the RAC GTPase family. Exons of RAC GTPases had their expression level in each dataset calculated in terms of RPKM, the number of RNAseq reads per million total reads per kilobase length of the exon. Exon RPKM in each cell type was then plotted against that cell type's expression level of canonical EMT markers. These results suggest that RAC splice isoforms with higher affinity for GTP or lower GTPase efficiency, both of which cause the proteins to spend more time in its active conformation, are more common in metastatic breast tumors. This underscores the importance of changes in cytoskeletal regulation in the progression of carcinomas and the potential for events other than coding mutations to have deep effects on tumor behavior.

Advances in Molecular Biology in Progression Towards Human Genome Engineering Therapies

Neil Mehta

Adviser: Adel Mahmoud

While genetic disorders are individually rare, many exist, and collectivelythese conditions affect millions of people. The lack of available treatments and cures for these conditions has resulted in hundreds of millions of dollars spent in caring for these patients each year globally. Gene therapy techniques were a first pass attempt at curing these diseases; clinical trials for gene therapy increased dramatically throughout the 1990s into the 2000s. Gene therapy methods, however, were risky due to 1) adverse effects genetic vectors used had on patients and 2) a lack of knowledge of the human genome and the adverse effects foreign DNA expressed in humans might have. Current advances to both viral and non-viral genetic vectors have reduced immunogenicity and increased delivered DNA stability in host tissues. Next generation sequencing has enabled rapid, inexpensive, and accurate whole-genome sequencing that is very useful for not only learning more about the human genome and genetic disorders but also for providing patients better and more targeted care in the clinic. The development of CRISPR/Cas9 genome editing methodologies have revolutionized the possibly surrounding genome therapies in humans. These dramatic improvements in molecular biology, genomics,and bioengineering research all point to a revolution in modern medicine.

Characterizing the lobule-specific contribution of the cerebellum to executive function in mice through reversible inactivation

Julia Metzger

Adviser: Samuel S.-H. Wang

Studies indicate that specific cerebellar lobules form reciprocal loops with neocortical regions associated with executive functions, yet the functional significance of these connections has not yet been explored. We hypothesize that disruption of these cerebellar regions would lead to disruption in one or more executive domains: (1) repetitive behavior, as measured through a grooming assay; (2) social interaction, as measured through a three-chamber test; (3) cognitive flexibility, as measured through a Y-maze swim task; and (4) anxiety/curiosity, as measured through an elevated plus maze. We achieved cell-type-specific inactivation of molecular layer interneurons in mice through a pharmacogenetic approach using Designer Receptors Exclusively Activated by Designer Drugs (DREADDs) via lobule-specific injections of construct AAV8-hSyn-hM4D(Gi)-mCherry. Experiments included two cohorts: (1) adult group, with surgery at P42 followed by acute inactivation prior to behavioral tests beginning at P56 via intraperitoneal injection of DREADD agonist clozapine-N-oxide (CNO), and (2) developmental group, with surgery at P21 and constant oral administration of CNO [vehicle: water] until behavioral tasks beginning at P56. In both cohorts, Lobule VI inactivation increased perseveration in the Y-maze task and prolonged length of time spent grooming, while social interaction in the three-chamber task was unchanged. In the adult group, inactivation of Crus II and Lobule VII decreased grooming time, did not affect Y-maze activity, increased social interaction in the three-chamber task, and increased time spent in the open arm of the elevated plus maze, suggesting a loss of behavioral inhibition. In the developmental group, Crus I inactivation increased Y-maze perseveration and grooming lengths and reduced sociability, suggesting cognitive impairment. We plan further experiments to obtain a detailed anatomical characterization of the inactivated cerebellar region, and to correlate these results with our observed behavioral effects through a generalized linear model.

Structural Characterization of MOV10 and ASCC1, key components of Endogenous mRNA-based Signaling Pathways

Mitchell Ng

Adviser: Alexei Korennykh

Various signaling pathways control the processing, turnover, and translation of messenger RNA (mRNA) and, by regulating gene expression, play key roles in proper stress and immune responses. Two such pathways are the OAS/RNase L and Non-sense mediated mRNA Decay (NMD) pathways. Activated by second messenger 2’5’ linked oligoadenylates (2-5As) synthesized by oligoadenylate synthetase (OAS), the endoribonuclease RNase L cleaves single strand RNA (ssRNA) as part of an anti-proliferative axis of the interferon response. The downstream effect of the OAS/RNase L pathway, the destruction of ssRNA molecules from both viral and cellular sources, plays an integral role in anti-viral and anti-tumor interferon activities. Another degradative mRNA pathway operating downstream or in parallel to the OAS/RNase L pathway, the NMD pathway eliminates mRNAs containing specific premature translation-termination codons (PTCs) through activation of the RNA-induced Silencing Complex (RISC).

Using X-ray crystallography, the first aim of our work is to characterize Activating Signal Cofactor Co-integrator 1 (ASCC1), a homolog of AKAP7, previously identified for its catalytic degradation of 2-5A. Our second aim is to characterize both the structure and binding mechanisms of MOV10, an ATP-dependent RNA helicase binding to 3’ untranslated regions (UTRs) of mRNAs and comprising the RISC. MOV10 activation has been associated with miRNA-mediated translational suppression and has previously been shown to inhibit retroviral and retrotransposal activity. If successful, we will perform biochemical enzyme kinetic studies to further elucidate the molecular mechanisms for MOV10 and ASCC1 association with target mRNAs and 2-5As respectively. Elucidating the structures of MOV10 and ASCC1 individually and associated with target molecules will (1) allow identification of evolutionarily conserved domains essential for proper function, (2) elucidate the binding and catalytic mechanisms of specific IFN-activated proteins, and (3) bring a whole further understanding of the downstream effects of mRNA degradative pathways.

Stress-Associated Epigenetic Moderation of Gene Expression: A Longitudinal Developmental Study

Beverly Nguyen
Lisa Schneper, Iulia Kotenko
Adviser: Daniel Notterman

Epigenetic modifications, such as DNA methylation, regulate gene expression in response to environment. Methylation of genes involved in the immune system and in the hypothalamus-pituitary-adrenal axis, a neuroendocrine pathway that regulates stress, has been associated with environmental stress. The primary aim of this study is to elucidate the effect of stress on DNA methylation patterns. An additional goal is to understand the effect of puberty on methylation sites. To address these, we analyzed genomic DNA samples from the Fragile Families and Child Wellbeing Study (FFCWS) using the Illumina® HumanMethylation450 bead arrays. The FFCWS follows a stratified sample of approximately 5000 children born between 1998 and 2000 to mostly unmarried mothers. The 524 samples analyzed include salivary DNA from 242 individuals at the age of 9 and the age of 15. Additional samples were selected to allow us to further investigate a correlation between stress and telomere length. Infinium® HD genotype data for these individuals was also obtained. To validate our samples, we imputed age and sex from our methylation and genotype data. Interestingly, the estimated sex differed from the expected for 27 of 47 total samples. Therefore, we performed additional validation of our samples using PCR amplification of SRY, a Y chromosomal specific gene. Differentially methylated positions associated with telomere length, age and environmental stress were also identified. Future work includes bisulfite pyrosequencing validation, RT-PCR of RNA and the use of luciferase reporter genes to demonstrate a functional effect.

Nodal induced MMP-2 Regulates Cell Migration to form Asymmetric Cardiac Tubes

Anna Niroomand
Daniel Grimes
Adviser: Rebecca Burdine

In vertebrates, a physically symmetric exterior masks the asymmetric patterning of organs along the left-right axis which is established during development. The formation of the heart is of particular significance as it undergoes numerous asymmetric morphogenetic events in the process of embryogenesis. A failure in the proper left-right patterning of the heart is a major cause of congenital heart disease in humans. Cardiac jogging, an early event in heart development that involves the elongation of the heart tube as it moves away from the embryonic midline to the left, is driven by asymmetric cell migration, left-sided cardiac cells migrating faster than right-sided cells. This results in the leftward positioning of the heart tube. Previous work has uncovered that the left side-specific signal Nodal, a member of the transforming growth factor beta family of ligands, acts as a key signal driving increased left-sided cell migration. It is proposed here that Nodal signaling induces matrix metalloproteinase 2 (MMP-2) expression in left-sided heart cells, which, via asymmetric degradation of the extracellular matrix, facilitates fast cell migration on the left side. A previous microarray and RNAseq study has identified MMP-2 as being upregulated by Nodal. To understand MMP-2's role in the heart, a number of experiments are being undertaken. Characterization of MMP-2 began with an examination of cardiac jogging laterality in wild-type, *no tail* mutants that exhibit bilateral rather than left side-specific Nodal signaling, and MMP-2-inhibited embryos. MMP-2 was also cloned in order to create a probe for gene expression analysis. Future plans include the characterization of MMP-2 function in cardiac development utilizing both *in situ* hybridization and immunofluorescence for gene and protein localization, respectively. Epistasis tests will determine the pathway on which MMP-2 acts in controlling cardiac cell migration. These experiments will result in an increased understanding of the role of MMP-2 in cardiac morphogenesis.

Purification of the PACT domain, a conserved centrosomal targeting motif in the microtubule-interacting protein AKAP450

Marina Nogueira
Rachel Kadzik
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Microtubules are essential components of the cellular cytoskeleton and play fundamental roles in cell division and intracellular transport. Centrosomes are considered the primary microtubule-organizing center (MTOC) and recruitment of the γ-tubulin ring complex (γTuRC) to the centrosome is necessary for promoting microtubule nucleation. However, the Golgi has recently been shown to also be a MTOC. The centrosomal- and Golgi-binding AKAP450 (A-kinase anchoring protein) is a large coiled-coil protein that interacts with a variety of proteins implicated in the microtubule nucleation process. However, little is known about which proteins AKAP450 directly binds to and the functions of these interactions. Specifically, how AKAP450 and its interacting partners contribute to microtubule nucleation at both the centrosome and the Golgi remains to be understood. Thus far, AKAP450 and its isoforms have never been purified and examined biochemically. I aim to dissect the function and mechanism of AKAP450 in microtubule nucleation. This summer, I began by focusing on the motif known as the PACT domain, which is found at the C-terminus of AKAP450 and is responsible for anchoring the protein to the centrosome. Four different constructs containing the PACT domain with fluorescent and affinity tags were cloned and purified. The purification of the protein construct with the best solubility was further optimized, thereby providing the first tool to study AKAP450 in vitro. Future work will include the construction and purification of recombinant protein domains of AKAP450 and investigation into the roles the various domains play in microtubule nucleation. Ultimately, understanding the functions of AKAP450 will help elucidate mechanisms of microtubule nucleation and general microtubule dynamics in the cell at both the centrosome and the Golgi.

The Characterization of RNase L cleavage targets through an RtcB Ligation Assay

Nana Kwaku Offei-Addo
Adviser: Alexei Korennykh

Activated downstream of viral double-stranded RNA detection in a cell, the endoribonuclease RNase L cleaves select single-stranded RNA in the cell by binding to uracil nucleotides in the RNA and cleaving between the next two nucleotides. This project aims to characterize RNase L cleavage targets and to better understand this substrate selectivity. RNase L will be activated in cells to produce fragments with a 5’ hydroxyl, that will be captured for sequencing using the *E. coli* RNA ligase RtcB which specifically ligates RNA with 5’ hydroxyl to the 3’ end of RNA bearing a 2’-3’ cyclic phosphate. After ligating the RNase L cleavage products to a linker of a known sequence, I will generate cDNA libraries for next generation sequencing. Aligning this data to the genome will provide precise RNase L cleavage sites that can be characterized to determine if RNase L has selectivity for specific regions in RNA. Understanding how RNase L selectively cleaves targets will help elucidate which mRNA RNase L regulates post-transcriptionally during normal cellular function and how a disease state like a viral infection may alter its function.

Quantitative classification of stereotyped behavior and aging profiles in *Drosophila melanogaster*

Mary Catherine M. O'Gorman
Daniel M. Choi
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Aging, or the changes manifested in an organism over its lifespan, leads to various forms of degeneration over time. Complex interactions among genetic, developmental, and environmental factors contribute to these observed changes. However, the intricate relationship between aging and behavior is not well defined due to limitations in previous methods of behavior analysis, which tend to incorporate observer bias. Given the variability of the behavioral effects of aging, the study of phenotypic changes over time is important for gaining a more complete understanding of the process of aging. Here we utilize a data-driven method of classifying and quantifying the behaviors in the fruit fly *Drosophila melanogaster*. The results of this method indicate that the temporal profiles of different stereotyped behaviors in *Drosophila* do not change in the same way. The strongly non-monotonic aging profiles reveal that behavioral aging is not simply a decline in function. Moving forward, we plan to study how aging profiles change with various interventions. In particular, we will examine the ways in which lifespan extension, caloric restriction, and neurodegenerative disease affect these behavioral profiles over time.

Characterization of the fibronectin and type I collagen matrix production in human lung fibroblasts

Arence Paasewe
Adviser: Jean Schwarzbauer

Type I collagen is a fibrous extracellular matrix (ECM) protein and the most abundant extracellular protein in our bodies. It provides tissues with strength and structure and is a support for cell adhesion and growth. Collagen polymerizes into fibers using fibronectin matrix fibrils as a template. Fibers are stabilized by covalent cross-linking by the extracellular enzyme lysyl oxidase (LOX). Skeletal abnormalities, cardiovascular defects, and fibrosis arise from deficiencies in type I collagen assembly. To understand how defects in collagen assembly cause disease, we need a better understanding of the steps in collagen polymerization. My goal is to determine the role that fibronectin matrix plays in this process. Since LOX is known to bind to fibronectin, we propose that LOX binding to fibronectin matrix provides sites for initiation of collagen assembly. In this study, WI-38 (VA-13) human lung fibroblasts were investigated in order to determine if they are a viable cell line for use in LOX experimentation. Although cell lines such as mouse embryonic fibroblast cells have already been characterized and are commonly used in ECM experiments due to their rapid cell growth and matrix formation, a human cell line would be preferable for LOX experiments since many more reagents have been produced specifically for use on human cells. Using western blot analysis, WI-38 (VA-13) cells were analyzed for the ability to produce collagen I and fibronectin. The results identified the presence of type I collagen and fibronectin within the cell culture medium. To determine whether the secreted collagen and fibronectin proteins were assembled into a matrix, immunofluorescence analysis of WI-38 (VA-13) cells grown on coverslips was performed using polyclonal anti-fibronectin antibodies and a commercially available monoclonal antibody against human collagen I. We found that when the cells were grown to high density, a fibrillar fibronectin matrix was readily detectable. However, collagen I fibrils were not detected in these cultures. Further experiments are needed to confirm that these cells do not assemble collagen I matrix by using other anti-collagen antibodies and to identify other human cells that can be used to study the role of LOX and fibronectin in collagen matrix assembly.

The impact of flow environment on biofilm structure and competition in *Pseudomonas aeruginosa*

Deirdre Ricaurte
Adviser: Bonnie Bassler

The biofilm mode of growth is a central part of bacterial ecology; by mediating group behavior, it allows individual cells to exert a large collective impact on their environment, and improves their robustness against numerous external perturbations. Though not yet broadly understood, feedbacks between microbial communities and their environments are thought to depend on the extracellular matrix – a complex mixture of polysaccharides, proteins, and DNA secreted by biofilm-dwelling cells. Recent literature has suggested that matrix production mediates antagonism against competing, matrix non-producing strains and species. Further, existing work has made it apparent that irregular flow profiles typical of natural environments can have a dramatic impact on matrix production and organization. However, whether or not an alteration in flow profile also impacts the competitive behavior linked with matrix production has not yet been studied. We examined the relationship between environmental flow profile and matrix-mediated biofilm competition using fluorescent strains of *Pseudomonas aeruginosa*, an opportunistic biofilm pathogen well known to thrive in the lungs of patients with cystic fibrosis. Strains differing in production of pellicle A, an elastic matrix polysaccharide that is critical for biofilm formation, were competed in microfluidic chambers bearing simple or complex flow profiles; biofilms were qualitatively and quantitatively analyzed using fluorescent microscopy and custom Matlab scripts. Results indicate that, in a simple flow environment, matrix production does confer a competitive advantage to *P. aeruginosa*. However, in a complex flow environment, matrix non-producing mutants establish a balanced mixture with producing strains. Flow velocimetry assays indicate that this competitive change is brought about by an alteration in matrix structure. Specifically, structural changes within the matrix induce surrounding microenvironments of slower flow, in which matrix non-producing mutants accumulate. Our results demonstrate the intimate links between hydrodynamic conditions, biofilm architecture, and population dynamics within bacterial communities.

Uncovering a Role for the Formin Protein mDia1 in Fibronectin Matrix Assembly

Maddy Russell
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Diabetic nephropathy is characterized by the buildup of extracellular matrix (ECM) within glomeruli of the kidney. Assembly of fibronectin, a principal ECM protein, has recently been shown to increase in high glucose conditions and may contribute to abnormal ECM accumulation. However, the mechanism by which this occurs is unknown. High concentrations of glucose are known to lead to the nonenzymatic modification of proteins to form advanced glycation endproducts (AGEs) and preliminary data show matrix assembly increases when cells are grown on an AGE-modified matrix. The formin protein mDia1 is associated with RAGE, the primary signaling receptor for AGEs, and is a main effector of RhoA GTPase, a required protein for matrix assembly. Therefore, mDia1 is a promising link between high glucose conditions, AGEs, and increased fibronectin assembly. To investigate the potential role of mDia1, we blocked its activity and analyzed the effects on the cytoskeleton, survival and matrix. Treatment of 3T3 fibroblasts with SMIFH2, an inhibitor of the conserved FH2 domain in the formin protein family, was found to disrupt the actin cytoskeleton. The inhibitor was also shown to have a cell density-dependent effect on cell growth, with cell death clearly present at 10μM and higher concentrations. Cells were able to survive for 24 hours at 5μM and this concentration can be used in matrix assembly experiments. These results confirm the importance of formin proteins in cellular architecture and survival. Cells grown in 5μM of the inhibitor showed no difference in matrix assembly after eight hours of treatment with SMIFH2. The lack of an effect on matrix assembly may be due to the instability of the inhibitor; future experiments will be performed at earlier time points to test the immediate effects of SMIFH2. The role of mDia1 in the molecular pathology of diabetic nephropathy remains promising and future experiments will more thoroughly investigate the effects of formin inhibition, and eventually mDia1 knockdown, on fibronectin assembly.

Characterizing the Roles of *Sec24CD* in Oocyte Determination and Maintenance in *Drosophila melanogaster*

Alexander V. Shahin
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A fundamental question in cell and developmental biology is how germline stem cells give rise to mature oocytes. However, the processes of oocyte determination and maintenance in *Drosophila melanogaster* are currently not well understood. To identify novel genes involved in oogenesis, an ethyl methanesulfonate (EMS) screen was conducted that revealed two mutant lines as having a ring canal clustering phenotype and missing oocytes. One of these lines, *JV91*, is an allele of *Sec24CD*, which encodes a protein involved in COPII-mediated vesicle transport. A cyst initially specifies an oocyte in *Sec24CDJV91* mutant egg chambers, but the oocyte reverts to a nurse cell fate by stage 4 of oogenesis. In addition, oocyte-specific factors such as Orb, Gurken, and Bicaudal-D accumulate diffusely at the ring canal cluster. To characterize the roles of Sec24CD during oogenesis, tagged transgenic lines containing wildtype *Sec24CD* were produced to visualize the developmental and cellular localizations of Sec24CD during oogenesis. *Sec24CD* mutant clones exhibit phenotypes similar to mutants in many *par*-related genes, which raises the question of whether Sec24CD is involved in polarity. Furthermore, since Sec24CD is part of the COPII complex, it would be interesting to determine whether oocyte determination is COPII dependent. Overall, understanding the role of Sec24CD in oogenesis will provide insight into the mechanism of oocyte determination in *Drosophila* ovaries, as well as potentially highlight new roles for the COPII complex in this process.

A Natural Microbiota of *Caenorhabditis elegans*

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A community of bacteria that colonizes another organism is referred to as its microbiota. Recent studies have implicated a dramatic impact of the human microbiota on the health status of individuals, including influences on metabolism, the immune system, and behavior. A highly tractable model system for researching the consequences of changes within the microbiota is lacking. Recently, *Caenorhabditis elegans*, a transparent soil nematode, has been used in microbiota research. *C. elegans* are typically maintained in the laboratory on monocultures of the bacterium *Escherichia coli*, which functions as a food source and also constitutes the microbiota. While this lab diet restricts the contact between microorganisms and the nematode to a single bacterial species, interactions between *C. elegans* and its natural environment are poorly understood. To inform future studies on *C. elegans* microbiota research, we have sought to define a natural microbiota for *C. elegans*. As *C. elegans* were originally isolated from rotten fruit, we grow sterilized *C. elegans* in compost samples to inoculate them with bacteria they could encounter in their natural habitat. After recovering the nematodes from the compost, we extract DNA and determine the identity of bacteria that have colonized their intestines by 16S rRNA sequencing. The nematode microbiota is compared to the bacterial community found in the compost sampled in parallel. We have sought to establish reliable methods for recovering *C. elegans* from compost samples and for extracting DNA from the bacteria that colonize a small number of nematodes. Once we define a natural microbiota of *C. elegans*, we will perform metagenomic analyses to assess interactions between *C. elegans* and its microbial colonizers. Further experiments may focus on manipulating the *C. elegans* microbiota to determine the impact of bacterial colonizers on the health of *C. elegans*.

Analysis of *Drosophila* Pico and its role in posterior signaling during oogenesis

Justin Song

Adviser: Gertrud M. Schüpbach

The *Drosophila* egg chamber consists of a monolayer of somatic epithelial follicle cells surrounding 16 germline cells. In stages 4-6 of oogenesis, the oocyte's EGFR signaling specifies the posterior follicle cells. Later, these PFCs produce an unknown signal that polarizes the microtubules of the oocyte leading to localization of *oskar* mRNA and Staufen protein. Prior literature has implicated the Pico gene in the regulation of actin cytoskeletal dynamics. Pico was found to mediate cell growth and proliferation at the tissue and organismal levels as reduced levels resulted in phenotypes with reduced growth, lowered cell division rates and increased G/F actin ratios.

In order to learn more about posterior signaling, I conducted experiments to characterize the role that Pico may play in the process. I quantified the mislocalization phenotype of Stau-GFP in Pico RNAi egg chambers and found that in many Pico RNAi egg chambers, Stau did not localize to the posterior in Stages 9 and 10. Therefore, Pico RNAi may disrupt the posterior polarizing signal. Since PFCs produce the posterior signal, Pico is expected to localize in the PFCs. Pico transgenes were used to determine the localization of Pico in the egg chamber. One fusion protein, Pico-GFP, showed that Pico localizes to the follicle cells. Kinesin is a motor protein that travels along the MTs of the oocyte cytoskeleton. Mislocalization of Kinesin in Pico RNAi suggests a disruption of oocyte MT polarity. I found that the percentage of Stage 9 egg chambers with Kinesin-lacZ mislocalization for Pico RNAi flies was much higher than for WT flies. Finally, correct apicobasal polarity is necessary for follicle cell signaling. Investigating the localization of polarity proteins such as aPKC may provide clues on the mechanism of Pico's involvement in posterior signaling. I used the Flp-Out technique to make mosaic egg chambers in which only some follicle cells express Pico RNAi. Anti-aPKC staining revealed that aPKC localization on the apical surface of follicle cells is not affected when Pico is knocked down. In conclusion, Pico is most likely involved in posterior signaling but future experiments especially with Pico mutants are needed for more information.

Type III Interferons Contribute to the Control of Flavivirus Infection *in vivo*

Yentli Soto Albrecht

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With a fatality rate of 20-50% and no effective antiviral therapies, yellow fever virus (YFV) is the prototypical hemorrhagic fever virus. However, its live attenuated form, YFV-17D (17D), is one of the most efficient vaccines ever developed. 17D differs from the wild-type strain by a mere 0.63% in nucleotide composition, yet both viral and host contributions to its attenuation remain elusive. *In vivo* work in knock-out (-/-) mice has recently highlighted the immunomodulatory role of host interferon signaling in controlling 17D infection. Interferons (IFNs), a family of widely expressed cytokines, are integral to the innate immune response and establishing an antiviral state in the host. Three types of IFNs have been identified: Type I (IFN α/β), type II (IFN γ), and type III (IFN λ). Although the role of type I and II IFNs is well described, the role of IFN III in controlling flavivirus infection remains to be fully understood. We characterized the phenotype of IFN λ, IFN α/β and IFN α/β/λ -/- mice. Our data show that IFN λ -/- display a wild-type phenotype, while IFN α/β/λ -/- exhibit a 30% fatality rate. Furthermore, clinical symptoms of disease were more severe and serum viremia higher in IFN α/β/λ -/- mice than in IFN α/β -/- mice. Currently, we are characterizing a 17D reporter strain to elucidate the impact of IFN λ on viral spread and tissue tropism via the *in vivo* spatial visualization of 17D during the course of infection. We believe that our approach will contribute to a better understanding of the role of IFN λ in regulating 17D pathogenesis—and, more broadly, to the identification of host factors governing flavivirus infection.

The Characterization of the Synthesis of Eicosanoyl-5-Hydroxytryptamide

Carolyn Stewart
Jennifer Bu
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The accumulation of heavily phosphorylated proteins within the brain has been shown to correlate with the onset of neurodegenerative diseases such as Parkinson's and Alzheimer's. Phosphoprotein phosphatase 2A (PP2A) normally dephosphorylates these crucial brain proteins. As we age, however, PP2A activity decreases, leading to an increased likelihood of developing neurodegenerative symptoms. Eicosanoyl-5-hydroxytriptamide (EHT), a component derived from coffee and recently found in mammalian tissue, has been shown to support PP2A activity, making it a promising therapeutic target in the prevention of neurodegenerative disease. This project seeks to uncover the enzyme responsible for the conversion of serotonin and eicosanoyl coenzyme A into EHT. Preliminary research using radioactive reagents and thin-layer chromatography suggests that the implicated enzyme is present within the bovine brain, though the presence of EHT product must be confirmed using high performance liquid chromatography or mass spectrometry. Future work will include purification of the target enzyme, followed by characterization of the enzyme's specificity, stability, concentration dependence, pH dependence, and kinetics. By understanding the function of this enzyme and beginning to manipulate it, the concentration of EHT may be indirectly controlled within the brain, leading to newfound preventative methods against neurodegenerative disease.

Functional Analyses of Reactive Oxygen Species (ROS) during Primordial Germ Cell (PGC) Development in *Drosophila melanogaster*

Sapna Syal
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REDOX mechanisms underlying generation of the Reactive Oxygen Species (ROS) have attracted considerable degree of attention due to both the deleterious as well as beneficial nature of effects induced by the reactive radical. In *Drosophila melanogaster*, ROS are thought to be crucial for the maintenance of progenitor states of immune cells in the lymph node. Moreover, a recent report has documented that ROS play a role in modulating the lifespan of germline stem cells (GSCs). Involvement of ROS in cellular proliferation, differentiation, and aging prompted us to explore the possible functional involvement of ROS in the development of embryonic Primordial Germ Cells (PGCs). We reasoned that since REDOX mechanisms have been shown to be involved in maintaining progenitor states, ROS could also be involved in PGC specification and behavior. Indeed, ROS can be detected in the embryonic PGCs from early stages of embryogenesis. Interestingly, we also observe a progressive increase in ROS levels in the PGCs as embryogenesis proceeds. Using both 'loss' and 'gain' of function mutations in different components of the REDOX pathway, we show that ROS activity/levels are likely to be important in maintaining germ cell identity and total germ cell count. Interestingly, changes in ROS levels influence the ability of PGCs to adhere to one another, suggesting potential involvement of this pathway in orchestrating different phases of germ cell migration. Experiments are underway to decipher the mechanistic underpinnings of different phenotypic consequences induced by changes in the ROS levels.

Characterizing the mechanisms of DNA sensor IFIX subcellular localization

Michelle-Ann Tan
Adviser: Ileana Cristea

Sensors of foreign DNA within the mammalian cell are essential to the intrinsic and innate immune defense against viral infection. A number of DNA sensors have been recently categorized as proteins within the PYHIN family, containing domains for homotypic protein-protein interactions and DNA binding. Of these, Interferon-Inducible Protein X (IFIX) is the most recently discovered and least understood. Previous findings show that IFIX can serve as a cytoplasmic DNA sensor despite being predominantly nuclear. However, the mechanism by which IFIX's subcellular localization is regulated so that it is available upon the entry of foreign DNA into the cytoplasm still remains uncharacterized. Therefore, this project aims to study the regulation of IFIX subcellular localization, specifically the mechanism by which IFIX is made available in the cytoplasm upon infection. By bioinformatics analysis, we identified four putative nuclear export signals (NES), NES1-4. Using direct immunofluorescence, we show that IFIX is able to colocalize with transfected vaccinia virus 70mers (VACV) in the cytoplasm despite the complete mutation of NES1, NES2, and NES4 by amino acid substitution. Further, IFIX in cells treated with nuclear export inhibitor LMB and/or protein translation inhibitor CHX also remains able to colocalize with VACV in the cytoplasm. Conclusions on the effects of protein translation inhibition cannot be made due to the lack of a CHX efficiency marker, but together, these results suggest that nuclear export is not required for the presence of IFIX in the cytoplasm upon viral infection. As such, we hypothesize that IFIX becomes available in the cytoplasm through protein translation and possibly a slow turnaround time by which IFIX moves into the nucleus after translation. We now pursue optimizing the drug treatment experiments to test this hypothesis. These studies will enable us to better understand how nuclear export and protein translation may affect the cytoplasmic localization of IFIX.

The Role of Jagged1/Notch Signaling in Breast Cancer Bone Metastasis

Rebecca Tang
Hanqiu Zheng
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Metastasis, the spread of cancer cells from original tumor to secondary sites, is responsible for over 90% of cancer deaths. Metastasis is organ-specific, guided by interactions between cancer cells and the metastatic tumor microenvironment. Over 70% of patients with advanced breast and prostate cancer develop bone metastasis.

In the normal context, the bone microenvironment is constantly being remodeled by osteoblasts, which are bone-forming cells, and osteoclasts, which resorb bone. During bone metastasis, this delicate balance is shattered; breast cancer cells tend to create osteolytic lesions. Targeting the bone microenvironment may interrupt metastatic progression of breast cancer cells; it is thus critical to understand the crosstalk between tumor cells at stromal cells at the bone metastatic niche.

Previous work in the lab pinpointed the critical role of Jagged1, a Notch receptor ligand, in mediating a vicious cycle of cellular signaling between breast cancer cells and the bone microenvironment that promotes metastasis. Here, we show that Jagged1 plays a significant role in mediating bone metastasis in the early stages of colonization. Furthermore, Jagged1/Notch signaling both confers chemoresistance and induced by chemotherapy. Finally, we demonstrate the efficacy of Jagged1 functional blocking antibody 15D11 in reversing these effects.

Investigating Mechanisms by which the Localization of Rac1b is regulated by Hypoxia

Daniel S. Thomson

Adviser: Celeste Nelson

Rac1b, a highly active splice variant of the small GTPase Rac1, is implicated in the progression of many human cancers. When Rac1b localizes to the membrane, it interacts with downstream effectors which can lead to cancer progression. Hypoxia, or the deprivation of adequate oxygen, is a characteristic of many tumors. It was recently shown that Rac1b translocalizes to the nucleus under hypoxia. We sought to confirm this result, and better understand the mechanisms underlying the translocalization. First, we found that Rac1b shows increased nuclear localization in low oxygen, confirming previous findings. We predicted two specific proteins may be involved in Rac1b translocalization. SmgGDS, a guanine nucleotide exchange factor (GEF) was shown to be necessary for Rac1b translocation. Interestingly, we found that SmgGDS has reduced expression under hypoxia. We also hypothesized that HMG-CoA reductase (HMGCR) may be involved in Rac1b translocalization. HMGCR is a critical enzyme in the mevalonate pathway, which synthesizes the prenyl group used by Rac1b for membrane targeting. To test whether HMGCR is involved in Rac1b translocalization, we treated cells with an HMGCR inhibitor. Treated cells had higher nuclear localization of Rac1b. Most importantly, there was no difference in localization between differing oxygen levels of cells with sufficient inhibitor treatment. We also found reduced HMGCR expression under hypoxia. These mechanistic insights for Rac1b translocalization may have important implications for understanding cancer progression.

Characterization of Mutations Associated with Constitutional Mismatch Repair Deficiency Syndrome (CMMRD)

Stephanie Tsai

Adviser: Alison Gammie

Constitutional mismatch repair deficiency syndrome (CMMRD) is a recessively-inherited childhood cancer predisposition disease characterized by a broad tumor spectrum that includes mainly hematological, brain, and colorectal malignancies. CMMRD results from either homozygous or compound heterozygous mutations in one of four genes (*MSH2*, *MLH1*, *MSH6*, or *PMS2*) encoding for proteins involved in DNA mismatch repair (MMR), a highly conserved mechanism that is critical for maintaining genome integrity. Due to the diversity in clinical outcomes of CMMRD and the relatively recent identification of the disease, CMMRD may be frequently mis- or under-diagnosed in the population. In addition, reasons for the wide variation in severity, age of onset, and clinical outcomes of CMMRD are currently not well-understood. In this study, it is hypothesized that CMMRD outcome is associated with the ability of the mutation to impair MMR function. By this model, less severe phenotypes result from mutations that confer residual MMR proficiency. We characterize CMMRD-associated mutations in terms of MMR ability using a *Saccharomyces cerevisiae* model. Using both recombination-mediated PCR-directed plasmid construction and integrated *delitto perfetto* approaches to site-directed mutagenesis, CMMRD-associated mutations are introduced in the cognate positions in the corresponding *S. cerevisiae* MMR genes. Qualitative and quantitative functional analyses of MMR ability are performed using canavanine counterselection assays. Decreased protein steady-state levels due to protein instability and inhibited protein-protein interactions will be examined as possible mechanisms for pathogenesis. Since CMMRD-associated malignancies are often difficult to treat using common DNA-damaging chemotherapeutic agents, we will further investigate whether the alleles confer differential drug resistance. Characterization of CMMRD-associated mutations and a clearer understanding of molecular mechanisms of CMMRD pathogenesis will have important implications for genetic counseling and the development of treatments and clinical guidelines.

The Role of Nucleotide Excision Repair and DNA Helicases in G-Quadruplex Processing in *Saccharomyces Cerevisiae*

Emilee Tu
Tom J. Pohl
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The preservation of genomic stability and integrity is essential for life. DNA is susceptible to damage which may arise from a variety of different agents, both exogenous and endogenous. R-loops, structures that form during transcription consisting of a DNA-RNA hybrid duplex and a displaced single-stranded DNA, and G-quadruplexes, G-rich nucleic acid sequences that can form four-stranded structures via non-canonical associations of guanines, are two endogenous structures that can cause DNA damage if not properly resolved. One mechanism for repairing DNA damage is nucleotide excision repair (NER). During NER, the lesion is excised and repaired by polymerase and ligase repair the excision. In a previous study, it was shown that R-loops are sensitive to NER. Because of structural similarities between R-loops and G4 structures, we propose that NER may also be involved in processing G4 structures. To test this hypothesis, we will perform direct repeat recombination assays to measure DNA damage in *Saccharyomyces cerevisiae* cells containing G4 motifs in the presence and absence of proteins involved in NER. If NER plays a role in processing G4 structures, we expect to see increased recombination rates at G4 structures in strains deficient for NER proteins involved. Similar assays can be conducted to analyze the roles of other helicases in G4 processing. This study aims to expand our understanding of G4 processing.

The Haze of Biopharmaceutical Drug Pricing

Bradley Thomas Wachtell
Adviser: Shirley Tilghman

The pharmaceutical and biotechnology industry has been under significant scrutiny for their drug pricing strategy. Branded drug prices rose almost 15% in 2015, following 2014 in which prices rose almost 10%. Furthermore, the industry has endured increased inquiry over the last year due to several cases of ill reasoned or extreme price gouging. The first of these was Turing Pharmaceuticals in which Martin Shkreli, a former hedge fund manager, bought a company which produced Daraprim and, immediately upon purchase, raised the price from $13.50 a pill to $750, more than a 5000% increase. There was also the case of Valeant Pharmaceuticals, a Canadian pharmaceutical company whose CEO recently resigned while under investigation by the Senate's Special Committee on Aging for a strategy in which the company acquired pharmaceutical companies or their drugs, often drugs that are older and off patent, only to significantly raise the prices of these medications. The issue of drug pricing has come to the forefront in the United States, where political pressure forced an 18-month investigation by the Senate Finance Committee into Gilead's pricing of Sovaldi and Harvoni, two drugs used to treat hepatitis C.This outrage over drug pricing in the Unites States is due to the fact thatAmericans pay more for prescription drugs than anywhere else in the world. This can be attributed to the fact that the United States government is not permitted by law to negotiate drug prices with pharmaceutical and biotechnology companies unlike the single payer insurance systems around the world. In order to understand why there is so much public scrutiny over the pricing of drugs the industry must be better understood. The distinction between biotechnology companies and pharmaceutical companies has been blurred in recent years as pharmaceutical companies used to be associated with chemically synthesized small molecule drugs and biotechnology companies with biologic drugs produced using living cells. However, recently this is not the case. Almost all pharmaceutical companies or their subsidiaries produce biologic drugs and many biotechnology companies produce small molecule drugs as well. Regardless, the global pharmaceutical industry was expected to generate $1.23 trillion dollars in revenue in 2014 and the biotechnology sector was expected to generate almost $300 billion dollars in worldwide revenue in the same year. In the United States alone brand name pharmaceutical small molecule drugs accounted for almost $164 billion dollars of revenue, generic pharmaceuticals small molecule drugs accounted for almost $43 billion dollars of revenue, and biotechnology biologic drugs accounted for almost $100 billion dollars of revenue. While there are vast revenues to be had in the pharmaceutical and biotechnology business, there are also enormous costs involved. In 2014 United States healthcare spending increased 5.3% to over $3 trillion dollars, almost 18% of the U.S. GDP, with prescription drug costs increasing approximately 12% to almost $300 billion, making prescription drug costs almost 2% of the U.S. GDP. Furthermore, as of 2012 the United States biopharmaceutical industry supported almost 3.4 million jobs for people working directly for biopharmaceutical companies as well as associated industries and companies such as vendor and supply chain companies.

To Test or Not to Test? That is (Not the Only) Question:
Public Attitudes towards Noninvasive Prenatal Testing

Sophie Wang
Adviser: Daniel Notterman

Advances made in noninvasive prenatal testing from fetal sex detection to whole genome sequencing hold the potential to induce a seismic shift in the landscape of genomic information, amplifying existing ethical and social concerns. Currently, conversations about uses of noninvasive prenatal testing as well as imaginings of its future largely exclude the voice of the public. This comprehensive exploratory study utilizes both qualitative and quantitative data to explore the public's interactions with noninvasive prenatal testing. I argue that members of the public should be brought on as stakeholders in dialogues regarding NIPT's future, because they can reveal key insights about NIPT's development and inform new directions for policymakers and healthcare providers.

GERM CELL GENOTOXICITY
THE LASTING EFFECTS OF PEDIATRIC CANCER THERAPY

Catherine Wetlinkski
Adviser: Daniel Notterman

In this thesis, I set out to examine fertility as a late effect of pediatric cancer therapy. I determine why fertility is an inadequate measure of the reproductive fitness of adult cancer survivors and explore the causes of deficits in fertility after pediatric cancer therapy. I focus mainly on the male germ cell line, exploring various sperm genomic damages that occur as a result of chemotherapy and/or radiotherapy in childhood and adolescence. Although sperm chromosome aneuploidy presents as a consequence of pediatric cancer therapy, I worry more with sperm DNA fragmentation, which includes single-strand and double-strand DNA breaks, which are genotoxic and have the ability to be transmitted to offspring. I find, however, that although DNA-impaired spermatozoa may fertilize an egg, it is unlikely for these damages to remain in the embryo or for the embryo to remain.

Assessing the Importance of Stem-Loop One in the Stability of and Target Regulation by Qrr4

Emily Wohl
Adviser: Bonnie Bassler

Quorum sensing is a cell-to-cell communication process performed by bacteria such as *Vibrio harveyi* to transition between individual and group activities. The pathway is initiated by changes in concentrations of extracellular molecules called autoinducers that induce a signal transduction cascade. Five Qrr small RNAs (sRNAs) lie at the heart of the signaling cascade; they function to establish gene expression patterns by controlling the translation of the mRNA encoding the transcription factors, LuxR and AphA. LuxR and AphA are two master regulators central in the quorum sensing circuit. The Qrr1-5 sRNAs are each predicted to contain four stem-loops. Previous work developed and used a method called RSort-Seq to determine the importance of nucleotides within the Qrr4 sequence. Surprisingly, this method revealed that individual mutations in stem-loop one (SL1) do not perturb Qrr4 function, even though SL1 has been proven to be critical for Qrr stability. Furthermore, mRNA targets that base-pair with SL1 have not previously been analyzed. To further understand the contribution of SL1 to Qrr4 function, we are extending the individual nucleotide analysis to understand its role in stability and base-pairing.

Differential expression analysis of PQM-1 homolog in humans

Kenny Wong

Adviser: Coleen Murphy

The study of longevity-associated genes is important for our understanding of aging and its associated symptoms in humans. Previous studies using the nematode *Caenorhabditis elegans*, which has a similar aging process as humans, have found that insulin/IGF-1 signaling activation is correlated with longevity. Specifically, activation of DAF-16 (human FOXO ortholog), a transcription factor downstream of insulin-like receptor DAF-2, extends life span in *C. elegans* by activating genes associated with stress response and longevity. A transcription factor PQM-1 has been found to have the opposite effect of DAF-16 by activating genes that induce development and aging factors. While a homologous component for DAF-16 in humans is known, a PQM-1 homolog has not been found. Collaborators with our lab identified several candidate genes of a possible PQM-1 homolog that encode proteins that bind to genes associated with shortened life span. One of these genes, znf-274, was shown to have the strongest correlation with the shortened life span phenotype. Our lab acquired human cell lines with differential expression of znf-274. We plan to identify the genes activated and inhibited in the samples through RNA-Seq analysis using differential expression analysis and alternative isoform analysis tools provided by R/Bioconductor packages DESeq2 and DEXSeq respectively. We hypothesize that the longevity-associated genes in our samples will display comparable levels of expression as the differential expression of pqm-1 in nematodes. Characterization of the possible pqm-1 homolog znf-274 will provide a better model for the mechanism of aging in humans.

The Etiology and Pathogenesis of Multiple Schlerosis

Wing Fei Wong

Adviser: Thomas Shenk

Multiple sclerosis (MS) is a chronic demyelinating immune-mediated disease affecting the nerves of the brain and spinal cord, resulting in the disruption of neural communication. This manifests clinically in a range of physical, mental and psychiatric problems, which are collectively group along five different phenotypic classifications. Globally, MS affects an estimated 2.5 million people, making it the most common autoimmune disorder affecting the central nervous system (CNS). While the mechanisms behind pathogenesis, including inflammation and demyelination, are more-or-less understood, the etiology of MS is unknown. Candidate causes range from genetics to infection, which bears significance to future research and treatment potential. This thesis will review the current literature concerning the four predominant groups of theories of MS etiology (genetic, infection, environmental, and diet); present original research on one particular theory: the Aze Hypothesis; and propose future directions for multiple sclerosis research.

Investigating the Role of EGFR Signaling in Regulating Class IV Dendritic Arborization Neurons in *Drosophila*

Derek Xu

Adviser: Elizabeth R. Gavis

Proper morphology of the dendritic arbor, the highly diverse and complex branched neuronal projections that receive inputs, is important for proper neuronal connectivity and function. Because defects in dendrite morphology have been implicated in neurological and neurodevelopmental disorders such as autism and schizophrenia, it is important to understand how dendrite morphogenesis is regulated to ensure proper dendrite morphology. Recent studies of *Drosophila* dendrite arborization (da) neurons have shown that dendrite-epidermis interactions play a role in determining dendrite morphology—for example, the microRNA *bantam* regulates epithelial signaling and endoreplication, coordinating synchronous growth of class IV da neurons and their substrate, the body wall epithelium; a recent study has also implicated that epidermal growth factor receptor (EGFR) is required for proper cutaneous innervation during development in mice. Here we investigate the role of EGFR in regulating dendrite morphology in *Drosophila*. Although neuronal expression of several EGFR ligands was previously found to be necessary for proper dendrite morphology, we were unable to verify those results; however, we found that knockdown of EGFR in either neuronal or epidermal cells results in increased dendritic branching. Finally, we have preliminary results that suggest that neuronal cytoskeletal rearrangement could explain the increased branching observed.

Protein Scavenging Flux is Variable and Elevated by Autophagy Knockdown

Kevin Zhang
Michel Nofal
Adviser: Joshua Rabinowitz

Pancreatic ductal adenocarcinoma (PDAC) is a lethal cancer frequently bearing mutations in *KRAS*, a regulatory gene that stimulates the uptake of whole extracellular protein via macropinocytosis. Through this process, known as protein scavenging, PDAC cells can supplement free amino acid supplies. Pharmacological inhibition of protein scavenging can starve tumor cells and block their growth.

While published research has shown that serum protein supplementation enables survival of pancreatic cancer cells in low amino acid conditions, quantitative measurements of the protein scavenging flux are lacking. We have developed a sensitive, quantitative assay for flux measurement based on an isotope tracer-based method and metabolic flux analysis. Using this assay, we have observed a large variation in the magnitude of protein scavenging performed by different cells.

To elucidate the genetic components of the protein scavenging pathway, we analyzed the results of a genome-wide CRISPR screen previously conducted in the lab, which identified genes associated with increased and decreased levels of protein scavenging. Interestingly, autophagy-related genes were prominent screen hits, and we hypothesized a competition between autophagy and protein scavenging. Using our protein scavenging assay, we have found a moderate elevation in the magnitude of protein scavenging performed by cells deficient for the autophagy genes *ATG5* and *ATG7*. Further research will explore the genetic nature of this interaction.

Effect of Environmental Stress on Growth and Telomere Length in *Schizosaccharomyces Pombe*

Terry Zhu
Lisa Schneper, Iulia Kotenko, Christopher Webb, Virginia Zakian
Adviser: Daniel Notterman

Telomeres are repetitive DNA sequences that prevent loss of genetic information and aberrant end-to-end fusions over successive courses of DNA replication. While telomeres gradually shorten under normal conditions, chronic stress has been shown to accelerate this process, a finding with broad social and clinical implications. However, a definitive molecular mechanism for stress-induced telomere shortening has yet to be established. In order to better understand this mechanism, we employed the model organism *Schizosaccharomyces pombe*, or fission yeast, which shares greater homology with human telomeres than *Saccharomyces cerevisiae*. We hypothesize that environmental stressors cause accelerated telomere shortening accompanied by transcriptional changes in telomere proteins, especially those involved in protecting or extending telomeres. We measured the effect of two environmental stressors, oxidative stress and nutrient deprivation, on growth by exposing fission yeast to hydrogen peroxide or removing nitrogen from the media. *S. pombe* exhibited inhibition of growth in response to both stressors, an effect which was dose-dependent in the H_2O_2 condition. Preliminary results suggest that neither nitrogen starvation nor H_2O_2-induced oxidative stress had an effect on telomere length. However, experimental optimization is still ongoing and these experiments must be repeated to ensure reproducible results. Future work includes examining the effect of additional stressors, as well as quantifying accompanying transcriptional changes in telomere proteins by analyzing RNA levels through quantitative reverse transcription PCR (qRT-PCR) and protein levels directly by western blot. We expect to observe increased rates of telomere shortening upon stress exposure, and the corresponding downregulation of proteins that protect and extend telomeres. These results provide both a model in which to study the relationship between stress and telomere shortening and further insight into the role stress pathways play in mechanisms that maintain telomere length.