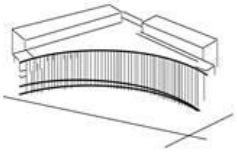
Department of MOLECULAR BIOLOGY

The Lewis-Sigler Institute for Integrative Genomics



Summer Undergraduate Research Program

Molecular and Quantitative & Computational Biology

Abstract Booklet

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Changes in Maternal Behavior Following Separations in Newborn Mice

Eman Ali, **Catherine Jensen Peña**

The behavior of the mother largely influences the development of newborn mice. Maternal separation of pups from the mother (dam) is a common model of early-life stress (ELS) in rodents. However, prior studies claim that maternal separation increases maternal care. We sought to resolve whether such changes in care are short or long-lasting over the course of the day, and therefore resolve whether increases in care could make up for the lack of care received during separation. Our study included 16 cages (7 standard-reared, 9 ELS-assigned). ELS lasted from P10-16, during which pups were placed in a separate cage from their dams for 3-4 hours per day. We observed all cages for 12 days (5 prior to ELS, and 7 during ELS), 3x daily (one hour in the morning, one hour immediately following reunion of the pups with their dam, and one hour approximately 2-3 hours after reunions), and made 20 behavioral observations during each hour. Behaviors such as nursing, pup-grooming, eating, drinking, and lack of contact were monitored. ELS increased nursing at all time points throughout the day, which may help compensate for pups' lack of nursing access during the maternal separation hours. In contrast, more active care behaviors such as pup-grooming, as well as total dam-pup contact time, increased only directly after the separations, suggesting that changes in dam behavior may not compensate for active and total maternal care induced by ELS. These results directly resolve controversy in the field, and confirm that the maternal separation model of ELS indeed disrupts overall offspring care.
This research was made possible by the generous support of the Lewis-Sigler Institute for Integrative Genomics.

Wiring receptor tyrosine kinases to user-specified pathways

Luke Barrett, Payam Farahani, **Jared Toettcher**

Epidermal Growth Factor Receptor (EGFR) is a receptor expressed on the cell surface. EGFR is activated by binding to ligands like Epidermal Growth Factor (EGF), causing it to phosphorylate tyrosine residues on itself and many other intracellular proteins to trigger a cellular response. The EGFR signaling network is complex, with many proteins recruited to the activated receptor, leading to activation of multiple intracellular pathways. This "fanning out" to multiple pathways makes the logic of the network quite difficult to decode. One solution to this challenge is to engineer a synthetic and controllable version of EGFR that only recruits and phosphorylates user-defined downstream effector proteins. Here, we present a synthetic EGFR with a modified C-terminal tail in which all tyrosines are mutated to serines, along with a specific tyrosine-based activation motif to recruit target proteins of interest. Preliminary findings indicate that our synEGFR does not recruit standard effectors of the Ras-ERK pathway, but it can selectively recruit fusion proteins containing the tandem SH2 domains of Zap70 (ZtSH2). This modular, synthetic receptor allows for the isolation of individual branches of the EGFR network and may serve as a new tool for synthetic gene circuit design.
This research was made possible by the generous support of the Lewis-Sigler Institute for Integrative Genomics.

Developing Artificial Soils for Myxococcus xanthus Locomotion Assays
Stephano Boyer-Paulet, Matthew Black, **Joshua Shaevitz**

Myxococcus xanthus is a soil-dwelling, swarming species of bacterium which moves via gliding motility. In swarms, the bacteria demonstrate rippling and fruiting body formation when in predatory and starvation behavior respectively. Their prevalence in soil and unique behaviors have made them an ideal model organism for studying soil bacteria cell behavior. However, experiments conducted thus far have mainly studied *M. xanthus* on gel plates and not their native soil. This has led to findings which only apply to two spatial dimension and ignore potential effects of a surface's curvature on bacterial behavior. To this end, we have used an artificial, hydrogel based, transparent soil which permits the use of confocal microscopy to study bacterial motility on a complex surface. Current findings suggest that *M. xanthus* don't distribute themselves according to a surface's curvature while in rich media. Successful future experiments utilizing this soil will allow for more robust models of *M. xanthus* behavior to be developed, improving understanding of soil bacteria behavior and cell-to-cell communication.
This research was made possible by the generous support of the Lewis-Sigler Institute for Integrative Genomics.

Identifying Binding Partners of the Dsl1 E Domain Using Proximity Labeling
Katherine Cappola, Halley Washburn, **Fred Hughson**

Multi-subunit tethering complexes (MTCs) help vesicles dock with and fuse to target membranes in eukaryotic cells. The simplest member of the largest class of MTCs is Dsl1, which facilitates vesicle docking and fusion in the retrograde protein trafficking pathway. Previous work has revealed one method Dsl1 uses to capture vesicles traversing this pathway: an unstructured region of Dsl1 known as the "lasso" binds to the vesicle coat protein COPI. However, additional research has suggested that another Dsl1 domain—the highly conserved "E domain"— enables vesicle tethering even when the lasso is absent. The mechanism by which the E domain tethers vesicles remains unknown. To clarify this mechanism, I aim to identify proteins that interact with the Dsl1 E domain *in vivo* using the proximity labeling technique TurboID. This project will help elucidate how MTCs latch on to specific transport vesicles, a process that promotes efficient intracellular trafficking.
This research was made possible by the generous support of the MolBio Summer Senior Thesis Fund.

SARS-CoV-2 Helicase Resistance to Nucleoside Analogs
Weston Carpenter, **AJ te Velthuis**

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the virus responsible for the coronavirus disease 2019 (COVID-19) pandemic. Replication and transcription of the SARS-CoV-2 genome is mediated by 16 highly conserved non-structural proteins (nsp), making them prime targets for antiviral drugs. Prior studies have demonstrated that the ability of the viral polymerase (nsp12) to use nucleotide triphosphates (NTP) for energy is inhibited by nucleoside analogs, compounds similar to NTPs. Subsequent research has also shown that mutations within nsp12's site of NTP catabolism can prevent binding of these drugs, resulting in viral resistance. However, little research exists studying the effects of nucleoside analogs on the viral helicase (nsp13) and whether this enzyme exhibits the same resistance. By purifying nsp13 and analyzing its unwinding ability *in vitro* in the presence of nucleoside analogs, I hope to determine whether these drugs have an inhibitory effect on the helicase as well. Furthermore, by selectively mutating residues within the NTP hydrolysis site of nsp13 to sterically inhibit nucleoside analog binding, I will investigate a potential resistance mechanism for the viral enzyme. Since the helicase is conserved across many other coronaviruses, research into its defenses against antiviral drugs could offer important insight into the general mechanisms of coronavirus resistance and aid the development of new antiviral therapies.
This research was made possible by the generous support of the MolBio Summer Senior Thesis Fund.

Investigating the role of the Argonaute protein Hrde-1 in the transgenerational epigenetic inheritance of *P. aeruginosa-14* avoidance in *C. elegans*

Jeremy Cha, Titas Sengupta, **Coleen Murphy**

For the nematode *C. elegans,* pathogens are a prevalent environmental stressor that can induce behavioral and epigenetic changes. Previous studies in our lab established that worms can learn to avoid pathogenic *Pseudomonas aeruginosa-14* (PA14) and then transmit this learned behavior to their progeny for four generations in a process known as transgenerational epigenetic inheritance (TEI). Our lab also demonstrated that the TEI of learned pathogenic avoidance is mediated by the small RNA (sRNA) P11 produced by PA14 and that neuronal TGF-β/DAF-7 signaling was required for the PA14 avoidance behavior. However, the additional endogenous molecular components and pathways involved in mediating the TEI of PA14 avoidance remain unknown. Hrde-1 is an Argonaute protein involved in nuclear RNAi in the germline. Preliminary data from our lab showed that loss of Hrde-1 reduces the PA14 avoidance behavior in worms. To characterize the behavioral and transgenerational effects of Hrde-1 on the TEI of PA14 avoidance, I will conduct behavioral choice assays using multiple generations of worms mutant for Hrde-1. To investigate the role of endogenous sRNA pathways in the TEI of PA14 avoidance, I will conduct additional behavioral choice assays with Hrde-1 mutants using PA14 that lacks the P11 sRNA necessary for the PA14 avoidance behavior. Furthermore, I will examine neuronal DAF-7 GFP expression in worms mutant for Hrde-1 to investigate the effects of Hrde-1-mediated pathways on the neuronal signaling that is required for TEI of PA14 avoidance. The results of this study will provide insight into the endogenous molecular pathways that mediate the PA14 avoidance behavior and TEI of the PA14 avoidance behavior in worms.

This research was made possible by the generous support of the MolBio Summer Senior Thesis Fund.

Investigating transcription factor FoxH1 in regulating TGFβ signaling in early heart development in zebrafish

Adrienne Chang, **Rebecca Burdine**

Left-right asymmetries arise from asymmetric expression of genes targeted by the Nodal and BMP signaling pathways and are implicated in morphogenesis and positioning of the vertebrate heart. Disruptions in asymmetric left-right patterning can result in congenital heart defects (CHD). In zebrafish, Nodal signaling in left-sided myocardial cells activates transcription factor FoxH1 and expression of Nodal targets, increasing cell migration rates. FoxH1 is also required for activation of BMP signaling in the endocardium that decreases cell migration rates in right-sided myocardial cells. However, the mechanism by which FoxH1 mediates endocardial BMP signaling to influence changes in myocardial cells is unknown. Since FoxH1 mediates Nodal signaling in myocardial tissue, I hypothesize that FoxH1 localizes in the myocardium to nonautonomously mediate endocardial BMP signaling. I plan to use triple fluorescent *in situ* hybridization to visualize FoxH1 in endocardial and myocardial tissue. I will then inject FoxH1 mutants with *foxh1* driven by tissue-specific promoters to rescue FoxH1 activity. This research will provide insight into how FoxH1 functions to regulate heart morphogenesis and establishment of left-right asymmetry to better inform us of the molecular basis of CHDs.

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

A novel chemical signaling system mediated by acyl-caprolactams in the human microbiome

Ye H. Chen, Luis Linares-Otoya, **Mohamed S. Abou Donia**

Quorum sensing (QS) is a mechanism of chemical signaling that allows bacteria to regulate gene expression and coordinate cell density-dependent behaviors. QS is crucial in the human microbiome, where quorum sensing signals, or autoinducers, regulate many host-bacteria processes that influence human health. However, many intercellular signaling systems in microbiome bacteria have yet to be characterized. Here, we identify a conserved three gene quorum sensing circuit in a biosynthetic gene cluster (BGC) in Bacteroidetes, a phylum highly prevalent in the gut microbiome. This circuit produces Acyl-Caprolactams (ACLs), which act as autoinducers by complexing with a sensor protein. By heterologously expressing truncated and complete constructs of the *Prevotella copri* ACL circuit in *Flavobacterium johnsoniae*, we show that ACLs upregulate expression of reporter genes. We find that *F. johnsoniae* expressing the circuit are unable to control ACL concentration and exhibit reduced growth, indicating the presence of additional mechanisms regulating ACL concentration in Bacteroidetes. We will also identify ACL-regulated genes and analyze the abundance and prevalence of the circuit to further investigate the role of the circuit in the microbiome. This may offer new insights into microbial interactions in the human microbiome, potentially contributing to the development of novel antimicrobial therapeutics.

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

Abstract

Chase Chiang, **Paul Schedl**

Eukaryotic chromosomes are folded into a series of distinct looped domains, termed topologically associating domains (TADs), in three dimensional space. A typical TAD is flanked by boundary elements (BEs). In addition to an architectural function, BEs function to insulate regulatory elements and/or genes in one looped domain from regulatory elements and/or genes. in neighboring looped domains. BEs in the Drosophila bithorax complex (BX-C) function to block crosstalk between adjacent segment specific regulatory domains that could disrupt expression of their homeotic genes and alter development. For example, the Fab-7 boundary blocks crosstalk between the iab-6 and iab-7 regulatory domains. iab-6 is turned on in A6 cells and it directs the expression of Abd-B in a pattern that specifies the A6 abdominal segment. iab-7 is off in A6 cells, but is turned on cells that give rise to the A7 abdominal segment and it regulates Abd-B in a pattern appropriate for the A7 abdominal segment. When the Fab-7 boundary is deleted, initiation elements in iab-6 inappropriately activate iab-7 in cells that should give rise to the A6 segment. As a consequence, A6 is transformed into a duplicate copy of A7. This change is segment identity can be observed in both homozygous and heterozygous Fab-7 mutants. In the former case, the transformation is complete, while in the later case it is not. The transformation in segment identity occurs at the blastoderm stage when the activity state of the BX-C regulatory domains (in this case iab-6 and iab-7) is set on or off by segment specific initiation elements. Once set, the activity state of the domain is remembered during the rest of development by a mechanism independent of the initiation elements. In the studies described here we have asked whether the activity of the Fab-7 boundary inherited from the mother and the father is equivalent at the blastoderm stage. For this purpose, we set up two crosses. In the first we mated WT mothers to fathers that are homozygous for Fab-7 deletions. In the second the mothers carried the Fab-7 deletion, while the fathers were WT. We find that the partial transformation of A6 to A7 in the heterozygous offspring is much more severe when the WT Fab-7 boundary is inherited from the father. This finding indicates that maternal and paternal chromosomes differ with respect to the establishment of a fully functional Fab-7 boundary.

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

Investigating the Role of the Methyltransferase SET-2 in the Transgenerational Epigenetic Inheritance of Learned Behavior

Maddy Chong, Titas Sengupta, **Coleen Murphy**

The ability to respond to pathogens is a core tenet of survival for all organisms. The nematode *Caenorhabditis elegans* consumes the virulent bacteria, *Pseudomonas aeruginosa* (PA14), upon first exposure but is able to protect itself by later learning to avoid it. Recently, the Murphy Lab discovered that this learned avoidance can be passed on to future generations via a novel model of transgenerational epigenetic inheritance (TEI). An important component in mediating the response to PA14 exposure is the COMPASS complex, a set of proteins conserved across species, including humans. It is responsible for genome-wide methylation, and loss of its catalytic subunit, SET-2, affects initial pathogen attraction and the subsequent learned response to the bacteria. To investigate the role of SET-2, a methyltransferase, I first confirmed the behavior of *set-2* mutants in response to PA14: mutants initially avoid the bacteria, unlike wild-type worms but do not develop additional avoidance after 24 hours of exposure. Next, I intend to rescue *set-2* mutants with tissue-specific expression of a wild-type copy of the gene to identify the tissue it acts in. I will then confirm that it acts as a part of the COMPASS complex using double mutants of *set-2* and other COMPASS complex proteins. Because it is an epigenetic regulator, I will also investigate how methylation by SET-2 affects transcription downstream. With these experiments, I seek to characterize the role of SET-2 in the PA14 response pathway, increasing our understanding of how learned behaviors can be propagated transgenerationally.

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

Novel Functional Characterization of Alternative Splicing of *TCAIM* in Breast Cancer

Allison Chou, Yong Wei, **Yibin Kang**

Cancer is one of the world's leading causes of death, with female breast cancer as the current leading cause of new diagnoses. Alternative splicing, a process that affects most human genes, is often dysregulated in cancer. In recent years despite the efforts to quantify alternative splicing in cancer patients using established transcriptome data, few studies have functionally characterized these identified, potentially important alternative splicing events in cancer progression. Further characterization of these events will reveal novel mechanisms of metastasis regulation and develop novel diagnostic markers and therapeutic options. Our preliminary computational work has shown that *TCAIM* (T cell activation inhibitor, mitochondria) is alternatively spliced in human breast cancer. The different isoforms of *TCAIM* are differentially expressed in tumor vs normal and among major breast cancer subtypes, indicating potential contributions to breast cancer tumorigenesis and/or cancer progression. We propose to characterize the functions of the short and long variants of *TCAIM* in breast cancer tumorigenesis, tumor progression and metastasis using lentiviral knockdown and overexpression systems. The study may determine the potential role of *TCAIM* and its splicing regulation in tumorigenesis and tumor progression.

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

Transmural Pressure and Retinoic Acid Signaling in the Mouse Lung Mesothelium

Nelson Chow, **Celeste Nelson**

Congenital diaphragmatic hernia (CDH) is a developmental defect characterized by a weak diaphragm that permits abdominal contents to spill into the thoracic cavity. Mechanisms by which this altered physical environment impedes cardiopulmonary development has been the subject of much investigation. Our lab has recently drawn a connection between the mechanical and biochemical pathways that regulate proper development of the lung by implicating the mechanosensor YAP as a mediator between transmural pressure (ΔP) and retinoic acid (RA) signaling in the epithelium and mesenchyme. Here, a combination of biochemical methods are employed to elucidate whether YAP serves an intermediary role in the lung mesothelium downstream of ΔP and upstream of RA signaling. Preliminary results show that, consistent with the proposed pathway, lungs cultured in XMu-MP-1 appear to have reduced RA signaling in the mesothelium. XMu-MP-1 is an inhibitor of MST1/2, an element upstream of YAP in the Hippo pathway. Unexpectedly, lungs cultured in Forskolin, a chemical agent that elevates ΔP, does not increase RA signaling. These conflicting implications for YAP as an intermediate in the pathway between ΔP and RA signaling in the mesothelium will be studied further moving forward. Findings from this study will improve our understanding of the etiology of CDH, which is important for identifying new treatment strategies.

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

Global Translation Quantification in Early *Xenopus* Development

Bhoomika Chowdhary, Alex Frese, Andrea Mariossi, **Martin Wühr**

Translational control of protein synthesis plays an important role in gene expression. At the cellular level, translational rates define the global proteome, which plays an essential role in directing intracellular processes, cell growth, and differentiation. Ribosome profiling becomes an important technique for quantifying translational efficiency when combined with RNA expression levels, and informs our understanding of global translation regulation when integrated with protein abundance measurements. By working to define the relationship between translational regulation and global protein levels over the course of embryogenesis in Xenopus laevis, we can learn more about the role of various proteins related to function, organization, and regulation of cellular processes important for development. Foundational objectives of this study include optimizing ribosomal profiling for Xenopus embryos to measure protein translation rates in early development. Preliminary results have elucidated lysis methods that are successful at isolating ribosomes from the embryos. This study aims to improve upon existing mass-action kinetics models to accurately predict protein dynamics during Xenopus embryogenesis using the initial maternal protein deposit in the egg, mRNA dynamics, and translational efficiency.

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

Creating an In Vivo System to Study Cell Cycle Regulation of Pluripotent Stem Cell Differentiation During Mammalian Gastrulation

Aaron Cohen, Rebecca Kim-Yip, **Eszter Posfai**

Gastrulation is a significant morphogenetic event in early mammalian development when precursor pluripotent epiblast cells migrate through the primitive streak and differentiate into mesoderm, endoderm, and primordial germ cells. The formation of these distinct cell types is required for the proper development of the organism's tissues, organs, and germline. Not only do epiblast cells migrate and differentiate during gastrulation, but they also rapidly proliferate. This suggests that careful coordination of these processes must exist to ensure the embryo develops with the proper cell number and types. Recent evidence in human pluripotent stem cells (PSC) suggests that cell cycle progression interacts with cell fate specification during gastrulation. However, a significant limitation of these studies has been the inability to create an aligned in vivo model. To investigate the cell cycle's role in coordinating differentiation, I will use PIP-FUCCI, a well characterized live reporter of cell cycle phase. I will study the cell cycle's role in coordinating germ layer and primordial germ cell specification in vitro using mouse embryonic stem cells. Then, I will create a reliable in vivo system via a transgenic mouse line to validate findings in a gastrulating mouse embryo. This summer, I have made progress on establishing a formative state PSC line that I will use to investigate cell cycle regulation of PSC differentiation during gastrulation. I have also made progress on characterizing and validating an in vivo reporter of PSC differentiation. My findings will expand our understanding of the mechanisms of cell cycle coordination of cell differentiation and germ layer formation during gastrulation and mammalian development.

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

Investigating the Role of E-cadherin in Epidermal Stem Cell Division Orientation

Audrey Czuchna, Brandon Trejo, **Danelle Devenport**

Epidermal stem cells balance renewal and differentiation through symmetric and asymmetric divisions to maintain their population while generating proper skin morphology and function. An imbalance in basal stem cell self-renewal or differentiation can result in cancer and tissue degeneration. However, the mechanisms that determine a stem cell's division orientation and fate to meet the tissue's needs remain unknown. Recent studies have demonstrated that differentiated suprabasal cell layers can regulate the replication of basal stem cells, but how this occurs is unknown. I hypothesize that the mechanosensitive adherens junctions between basal and suprabasal cells play a role in directing the division orientation of epidermal stem cells. To investigate the role of apical adherens junctions in basal stem cell division, I have conducted immunofluorescence assays on the basal cells within the epidermis of E-cadherin conditional knock-out mice. Using confocal microscopy, I have found that the loss of E-cadherin within basal cells impedes proper adherens junction formation, resulting in a decreased amount of α-catenin at the lateral cell border. Additionally, I show that loss of E-cadherin inhibits perpendicular basal cell divisions. This research will reveal new insights into the role of adherens junctions and E-cadherin on epidermal stem cell divisions, further expanding our understanding of the core processes behind wound healing and developmental skin abnormalities.

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

Understanding the role of Hh signaling during embryonic germ cell migration in *Drosophila melanogaster*

Juliana DaSilva, Girish Deshpande, **Paul Schedl**

In Drosophila melanogaster, the embryonic gonad is made up of primordial germ cells (PGCs) and somatic gonadal precursor cells (SGPs). As the PGCs and SGPs are specified at distant locations, gonad formation involves directed migration, recognition and sustained association between these two cell types. Specification of SGPs depends on the action of zygotic patterning genes whereas PGCs are formed under the control of maternal determinants at the blastoderm stage. PGCs formed on the external surface, are internalized by the midgut invagination and after making their way through the midgut they migrate through the mesoderm towards the SGPs. Their precise trajectory is governed by repulsive cues which are generated by Wunen(s) (lipid phosphate phosphatases) and by attractive cues, produced and interpreted by a non- canonical Hedgehog (Hh) signaling pathway. Studies over the last two decades have identified factors, like HMGCoA-reductase (Hmgcr), that specifically potentiate the transmission of the Hh ligand and have connected its reception by PGCs to a downstream GPCR, Trapped in endoderm (Tre1) that remodels the cytoskeleton to promote directed migration. Further supporting these claims here, we show that: I) Hh functions in the embryonic mesoderm to attract PGCs throughout migration. II) Hmgcr is also required in the mesoderm to potentiate the chemoattraction and III) Mesodermal loss of either Hh or Hmgcr leads to germ cell migration phenotypes that resemble that of complete loss of tre1. Lastly, our data demonstrate that Hh and Hmgcr function in a cell autonomous manner to guide germ cells.

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

Quorum-sensing-mediated communication between phage VP882 and its bacterial host informs phage decision-making

Evan DeTurk, Grace Beggs, **Bonnie Bassler**

Quorum sensing (QS) is a process of bacterial cell-to-cell communication facilitated by extracellular signaling molecules called autoinducers (AIs). AIs are synthesized by bacteria that use them to orchestrate collective behaviors through group-wide detection. In *Vibrio cholerae*, one QS pathway consists of the receptor VqmA and the AI 3,5-dimethyl-pyrazin-2-ol (DPO). The vibriophage VP882 encodes a VqmA homolog ($VqmA_{Phage}$) that binds DPO. $VqmA_{Phage}$-DPO binding is used in the phage VP882 lysis-lysogeny decision making process, meaning that the phage exploits host-derived information to modulate its own behaviors. It is not fully understood how $VqmA_{Phage}$ competes with its host for DPO binding to induce lysis. Uncovering this information is important for the development of broad-spectrum phage therapy based on phage VP882 and understanding how phages incorporate host-derived information into their behaviors. We have purified $VqmA_{Phage}$ and begun to assess its DPO-binding characteristics. We plan to define how $VqmA_{Phage}$ competes with its host for DPO binding and characterize the overall binding specificity of $VqmA_{Phage}$. Additionally, the VP882 gene *gp60* is predicted to encode an important regulator of the VP882 lysis-lysogeny transition. We plan to uncover the function of *gp60* by assessing its influence on host lysis and identifying its downstream regulatory targets.

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

An Investigation of the Role of Chronic Stress on Mitochondrial Functioning in the Mouse Brain

Varun Devraj, Eugenia Xu, **Daniel Notterman**

Chronic stress can lead to adverse health effects, including increased rate of aging, brain damage, and cardiovascular disease. On an intracellular basis, the stress response has been recently linked to dysfunction of mitochondria. Indeed, it has been reported that chronic stress leads to impaired enzymatic activity of isolated electron transport chain (ETC) complexes and increased production of general free radicals in mouse mitochondria. However, the precise impacts of chronic stress in the brain on physiologically relevant mitochondrial parameters, such as coupled respiratory capacity, mitochondrial membrane potential, and specific free radical production, are unknown. Using brain tissues from the chronic restraint stress (CRS) mouse model, we hypothesize that CRS will lead to compromised mitochondrial respiration and increased nitric oxide (NO) production, reflected in differential expression of the genes linked to these functions in the brain. To characterize mitochondrial function under chronic stress conditions, we will utilize both a fluorescent probe of mitochondrial membrane potential, as well as measure oxygen consumption of coupled, respiring mitochondria in live time. In parallel, we will conduct expression analysis of genes related to mitochondrial functioning and dynamics to establish a basis by which chronic stress mediates mitochondrial dysfunction. By correlating this differential gene expression to mitochondrial respiratory parameters, we hope to provide a greater understanding of the mechanism by which mitochondrial dysfunction is implicated in the brain's stress response.

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

Identifying Novel Pyrenoid Tubulogenesis Candidates

Keenan Duggal, Eric Franklin, Ed Cruz, **Martin Jonikas**

In the coming decades, an increase in crop production efficiency will be needed to offset the strain of human population growth and climate change on global food supplies. Carbon fixation – the natural photosynthetic process of converting atmospheric carbon dioxide (CO_2) into usable biomass – is the rate limiting step for modern crop growth. Consequently, optimizing the process of carbon fixation is an effective and ecofriendly pathway towards achieving higher crop yields. An elegant solution to this challenge comes from evolutionary adaptation in *Chlamydomonas reinhardtii. C. reinhardtii* is a species of algae with a chloroplast that contains a specialized subcompartment, the pyrenoid, which has evolved to efficiently concentrate and fix CO_2. Bioengineering the pyrenoid into staple crops could greatly improve their photosynthetic efficiency, and therefore their net yields. Presently, the genes associated with some pyrenoid components are characterized, but very little is known about the mechanisms of tubule formation. Here, I aim to identify proteins and lipids involved in pyrenoid tubulogenesis. I have performed a modified co-immunoprecipitation assay and conducted a compositional analysis on isolated, intact thylakoid membrane fragments which has yielded several promising protein candidates specifically enriched in the tubules. Additionally, I have performed several experiments to optimize the assay in order to produce deeper and cleaner proteomic data. Using this optimized procedure, I will develop a comprehensive pyrenoid tubule proteome. Next, I will generate knockout mutants for interesting candidates to determine their role in tubulogenesis. This work will advance our understanding of the pyrenoid, and move towards bioengineering the entire structure into land plants.

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

Towards RNA Antibiotics: Investigating antisense RNA Targeting *Escherichia coli* genes for Bactericidal Effects

Minatulah Elzawawy, Jiayi Yuan, Jake Margoi, **Zemer Gitai**

The rise in multi-drug resistant pathogens caused by the dissemination of resistance genes, combined with the shortage of new antibiotics highlights the need for alternative therapeutics. Narrow-spectrum, species-specific antibiotics are a promising solution that offers a way to prevent harm to endogenous host microbiota and combat the spread of resistance genes. To design species-specific antibiotics, the use of programmable antisense oligomers, which have complementary nucleotide sequence to target mRNAs, has previously been proposed. Using antisense RNAs (asRNAs), this study seeks to design constructs that decrease target gene expression and ultimately kill bacteria. Preliminary results show that CRISPRi knockdown of *parC* and *parE*—which encode topoisomerase IV subunits that unlink daughter chromosomes during segregation—kill bacteria and result in growth defects, respectively, making them candidates for antisense targeting. In this study, antisense constructs targeting the start codon of *parC* and *parE* genes—which is recognized by ribosomal components during assembly of the translation apparatus—to block translation initiation were found to decrease the growth of bacteria. However, the search for bactericidal constructs continues. As previous studies have demonstrated, rational design strategies have not always been successful at producing constructs that decrease target gene expression and kill bacteria. Thus, this study proposes the use of a random asRNA expression library with the potential to target diverse transcripts in order to conduct an unbiased screen for bactericidal asRNAs and ultimately identify the corresponding gene targets. With these findings, I will further investigate the antisense mechanism of action by using RT-qPCR to assess whether mRNA decay occurs, and by integrating the *GFP* gene downstream of the putative gene targets to measure fluorescence as a proxy for protein expression to confirm that asRNAs inhibit translation.

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

Investigating Novel Digoxin Metabolism by the Human Gut Microbiome

Julia Garaffa, Catherine Day, Ruojun Wang, **Mohamed Abou Donia**

The composition of the gut microbiome has a significant impact on health outside of the digestive system. More than 50 drugs have been identified to undergo microbiome-derived metabolism (MDM). MDM is a process in which ingested drugs are modified by flora in the gut microbiome to form a metabolite product, which may act differently from the original drug. One such MDM positive drug is digoxin, which is used to treat heart failure. Digoxin is metabolized into an inactive form by the bacterium Eggerthella lenta via the *cgr* gene operon. However, a previous screen produced three microbiome samples in which digoxin was metabolized, but *E. lenta* was absent. Therefore, the ultimate goal of this research is to identify the causative agent of digoxin metabolism in these samples. First, we will conduct a search to determine if *E. lenta*, *cgr2*, or a homolog thereof is present in the samples. If *E. lenta* is confirmed to be absent, the causative agent responsible for digoxin metabolism will be identified through MDM-Screens, functional metagenomics screenings, and comparative RNA sequencing. Thus far, metagenome analysis has shown that presence of *cgr2* does not accurately predict digoxin metabolism, which is in disagreement with previous literature. This research may unveil a previously unknown mechanism regulating digoxin metabolism or a new organism capable of metabolizing digoxin. We expect that the outcome of this research would lead to developing microbiome-based screening procedures that allow for more personalized digoxin treatments.

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

Functional testing of rapidly evolving regulatory elements across gliding marsupials

Bryan Guevara, Jorge Moreno, **Ricardo Mallarino**

A fundamental challenge in evolutionary-developmental biology is to understand how changes in the genome can lead to phenotypic differences within and between species. Morphological structures which have evolved independently in closely related species provide a particularly powerful framework for addressing this question, as such traits may have arisen through similar coding or regulatory changes. The gliding membrane, a specialized adaptation that allows gliding mammals to propel as they fall through the air, serves as a useful model as it has independently evolved across many taxa, including three closely related marsupial species (*Petauroidea* superfamily). Previous work has implicated Emx2, a transcription factor, as important for correct patagia development and has uncovered putative regulatory elements which both seem to be regulators of Emx2 and are evolving rapidly in gliding species. Here we aim to functionally test the ability of these bioinformatically predicted cis-regulatory elements to function as regulatory elements and to assess the effects of acceleration on the elements' ability to regulate expression. To test these, we will conduct a luciferase reporter assay, this assay will inform if these elements potentially function as regulators of Emx2, if nucleotide changes found confer differential regulatory activity, and if these elements undergoing selection in one species work with elements from different species. Taken together the results of this assay will ultimately confirm our bioinformatic prediction of cis-regulatory elements and provide insights into how gliding phenotypes may have arisen through differentially evolved genomic changes which target the same regulatory targets. Furthermore, this study provides a strong basis for understanding how evolutionary genomic changes alter subsequent transcriptional/phenotypic outputs within and between species in the *Petauroidea* superfamily and may provide translational knowledge for other species in similar circumstances.

Connectomics analysis of neural thermosensory and behavioral pathways underlying decision-making in Drosophila

Judah Guggenheim, Max Aragon, **Mala Murthy**

Natural environments contain multiple complex streams of sensory information. To produce appropriate behaviors, animals must decide what information to focus on and what information to ignore in a given moment. How this decision-making process is implemented in the brain is poorly understood. Behavioral experiments in the fruit fly *Drosophila melanogaster* suggest that during courtship with a female fly, male flies opt to either "stay" or "switch" in response to a heat pulse. During a "stay" decision, flies effectively ignore the heat and continue courting, while during a "switch" decision, male flies move away from the female and terminate courtship. We hypothesized that these stay/switch decisions may be implemented in the brain through GABAergic inhibition of neurons within the fly's heat-sensing neural pathway. To this end, we identified GABAergic neurons that synapse onto first-order, second-order, or third-order neurons in the heat pathway using FlyWire, a human-AI generated platform employing electron microscopy to display the Drosophila connectome. Preliminary findings indicate that inhibition onto first-order thermosensory neurons (hot cells) and second-order neurons (projection neurons) comes mostly from neurons with neurites confined to the antennal lobes. Inhibition onto third-order neurons in the heat-sensing pathway comes from diverse neuropil regions, including regions enriched with sexually dimorphic *doublesex*-expressing neurons involved in courtship behavior. These findings suggest that distractor information may be preserved in early sensory areas regardless of the behavioral decision outcome, and that courtship-related neurons may coordinate inhibition onto higher-order neurons in the heat-sensing pathway that generate escape behaviors. Future behavioral experiments will examine the extent of distractor preservation in the brain by testing if male flies can learn to associate heat with a visual landmark during courtship.

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

Astrocyte Interaction with the Extra Cellular Matrix

Jacob Hayes, **Jean Schwarzbauer**

Extracellular matrix (ECM) proteins play a central role in both non-neuronal wound healing and central nervous system (CNS) recovery. In both processes, the injury progresses from a plasma derived fibrin (FB) clot to a fibronectin (FN) matrix to a scar matrix rich in collagen; however, this scar causes problems in the CNS, preventing complete recovery. This pertains especially to one cell type, the astrocyte, which far outnumbers neurons and are instrumental in the formation of the final scar. CNS recovery appears to be encouraged by early ECM proteins but discouraged by others later, so we suspect that the interactions of astrocytes with the ECM through different stages influence recovery before forming the final scar. To test this, four ECMs will be created, representing the early clot (FB), the provisional matrix (FB and FN), the transitional matrix (FB, FN, and collagen), and the fibrous glial scar (FN and collagen). So far this summer, methods have been standardized for creation of the first two matrices. Using fibroblasts, a known matrix interacting cell type, as control, these methods have been confirmed to allow cell attachment and staining/imaging of cells seeded on the first two matrices. Next steps include methods for creating the third and fourth matrices as well as confirming adhesion of fibroblasts to these matrices. This system will further be used to test astrocyte activity on the ECMs of CNS recovery to discover which ECM substrates may affect CNS recovery most.
This research was made possible by the generous support of the Susan W. and James C. Blair '61 P87 Endowed Senior Thesis Fund.

Abstract

Shelley Heo, **Zemer Gitai**

Bacteria can have heterogeneous behaviors in response to various stresses, which can lead them to adapt to stressful environments like antibiotic treatment. In the past, studies were limited to investigating heterogeneity a gene at a time, but now through single cell RNA-sequencing, we can assess heterogeneity for all genes in *Bacillus subtilis* and found that genes involved in prophage induction are expressed heterogeneously in *B. subtilis* treated with ciprofloxacin. Prophage induction has been well-studied in the model bacterium *E. coli* but not as intensely observed in bacterium like *B. subtilis* that is known to have multiple active prophages. In hopes of narrowing the gap in knowledge on prophage induction, this study observes prophage induction dynamics of *B. subtilis* in response to different stressors and will provide insight into the mechanisms of prophage induction. This will be done by profiling prophage induction through imaging with reporters and through ddPCR, and by conducting arrays of knockouts and assays of prophage induction. As preliminary results suggest that *B. subtilis* under DNA damage heterogeneously induces prophages, I hypothesize *B. subtilis* has varying prophage induction dynamics in response to different magnitudes and types of stressors. Results of the research will help contribute to our knowledge of prophage induction in bacteria and its general mechanism, which may allow us to develop antimicrobials in the future that take advantage of the pre-existing prophages in the bacteria to kill the cell.
This research was made possible by the generous support of the Susan W. and James C. Blair '61 P87 Endowed Senior Thesis Fund.

Developing a Cohort of Transgender Youth to Assess Psychological Well-Being and DNA Methylation During Gender-Affirming Care

Gillian Hilscher, **Dan Notterman**

Transgender youth (youth whose gender identity does not align with their sex assigned at birth) are at a higher risk for serious mental illness than their cisgender peers, largely due to gender dysphoria—the distress resulting from such a misalignment. Survey responses have shown that these adverse mental health outcomes are significantly ameliorated after receiving gender-affirming care in the form of puberty blockers or gender-affirming hormone therapy. However, stress biomarkers that corroborate these psychological findings—such as epigenetic aging—have yet to be analyzed. Therefore, this study aims to provide biologically based support for the mental health benefits of gender-affirming care by evaluating DNA methylation aging in peripheral blood mononuclear cells (PBMCs) of transgender youth as they receive treatment. While this study is in the recruitment stage, preliminary analyses reveal that the demographics of this cohort (n=16) are comparable to those of other studies, which supports the generalizability of future findings from this cohort. Not only will this study extend current understandings of general methylation changes during gender-affirming medical treatment, but it also has the potential to provide biologically based support for the mental health benefits of gender-affirming care through its analyses of epigenetic aging, a known indicator of biological stress.

This research was made possible by the generous support of the Crecca '46 Molecular Biology Senior Thesis Fund, NJ ACTS, the Rutgers Pediatric Clinical Research Center, and the Notterman Lab of Princeton University.

Rewiring cytokine receptors for custom cellular responses

Rachel Hsu, Emily Mesev, Payam Farahani, **Jared Toettcher**

Engineered cell-based therapies are a rapidly advancing technology that have much potential in treating a wide range of diseases. However, strategies for optimizing synthetic receptors as tools to tailor cellular outputs to customized inputs are urgently needed. Here, we propose a receptor biosensor platform based on cytokine receptors. Cytokine receptors use the simple, compact Janus kinase (JAK)-signal transducer and activator of transcription (STAT) pathway and thus provide much flexibility and can be used as a model to understand possibilities for synthetic receptors. We investigated the modularity of cytokine receptors as a synthetic platform by reprogramming type I and type III interferon (IFN) receptors to phosphorylate alternative STATs and novel substrates that can trigger user-defined responses. Preliminary findings indicate that cytokine receptors can be modified to sense other molecules, such as erythropoietin (EPO), and activate different substrates, including a phosphorylated tyrosine tag (pYtag) in the form of an immune tyrosine activation motif (ITAM). Activation of this pYtag triggers the recruitment of a cognate tandem-SH2 domain that can be conjugated to a fluorophore for detection. We further developed the IFN biosensor system design to trigger cleavage by a protease, which can potentially induce the nuclear localization of user-specified transcription factors. By implementing these receptors in human embryonic kidney 293 cells, we hope to create customized biological functions. This could potentially be a powerful tool for developing better synthetic signaling platforms and could have many therapeutic applications.

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

Determining the Protein Domain of Celsr1 Responsible for Vangl2 Junctional Recruitment

Ishea Johnson, Audrey Goh, **Danelle Devenport**

Despite advancements in prenatal care, neural tube defects (NTDs) continue to be one of the most prominent and fatal disorders affecting fetuses. While dietary insufficiencies have been shown to affect proper neural tube closure, it is likely that there is a genetic, heritable trait that impacts disordered neurulation. The planar cell polarity (PCP) pathway plays a fundamental role in ordering cells across a tissue, and mutations in PCP components have been implicated in cases of human NTDs. One such protein is Vangl2, which associates with the atypical cadherin Celsr1 to asymmetrically localize to intercellular junctions. Although PCP is instrumental in many embryological processes, the nature of the association between Vangl2 and Celsr1 in vertebrates is still largely unknown. To investigate the hypothesis that Vangl2-Celsr1 interaction is mediated through transmembrane and/or cytoplasmic regions, chimeric Celsr1 proteins in which the extracellular, transmembrane, or cytoplasmic regions have been swapped with that of E-cadherin were co-transfected into mouse keratinocytes with WT Vangl2. The results show that the absence of Celsr1's cytoplasmic domain results in lower cell-cell junctional recruitment of Vangl2. The next steps of this project are to create chimeras for Vangl2 using a tetraspanin not implicated in the PCP pathway to conduct similar border recruitment assays and determine the Vangl2 domain required for border recruitment.

This research was made possible by the generous support of the Envin `62 Thesis Fund.

Modulation of Immune Cell Migration and Cytokine Release by Marsupial Cathelicidins

Aellah Kaage, Jongbeom Park, **Ricardo Mallarino**

To counter bacterial threats immediately after birth, marsupial neonates rely on powerful innate immunity factors, as they are incapable of mounting an adaptive immune response. One set of such factors are the cathelicidins, peptides which can disturb bacterial membranes and interact with immune cells. Both of these roles have been well-documented in study of the singular human cathelicidin, but study of the high-copy-number marsupial cathelicidins has been focused primarily on direct antibacterial action. Work at the Mallarino and Donia laboratories has previously found putative cathelicidin-coding genes in the sugar glider (Petaurus breviceps), synthesized versions of which have shown antibacterial action. Our research aims to characterize the immunomodulatory effects of sugar glider cathelicidins, specifically regarding chemotaxis and cytokine release, by performing transwell migration experiments and enzyme-linked immunosorbent assays on mouse-derived immune cells treated with cathelicidins. We have observed varying effects across cathelicidins on cytokine release, as some cathelicidins appear to reduce secretion of the pro-inflammatory cytokine TNF-alpha, while others have no effect. The results of these and further experiments may clarify synergistic or specific properties of glider cathelicidins as they relate to protection of the underdeveloped young, as well as broaden the field's understanding of immune cell migration and inflammation.

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

Investigating the Post-Transcriptional Regulation of *dand5* RNA during Left-Right Patterning in Zebrafish

Maryam H. Kamel, Triveni Menon, **Rebecca D. Burdine**

Most vertebrates appear bilaterally symmetric; however, their visceral organs are left-right (L-R) asymmetric in structure and position. Improper L-R asymmetry leads to a spectrum of congenital disorders, including congenital heart disease. Thus, understanding molecular and cellular mechanisms that determine left-right axis during early embryogenesis is necessary. Fluid flow induced asymmetric expression of *dand5* mRNA in vertebrate embryonic left-right organizers (LROs) is the earliest known molecular asymmetry during L-R patterning. However, the exact mechanism by which *dand5* is regulated in response to flow remains unknown. This study investigates the post-transcriptional regulation of *dand5* RNA in zebrafish LRO, Kupffer's Vesicle (KV). Preliminary results show that *dand5* UTR is sufficient for its asymmetric expression in KV. To identify specific regulatory sequence elements, ~100 bp domains within the *dand5* 3'-UTR were deleted using designed gRNAs and Cas9 mRNA injected into zebrafish embryos. Injected embryos were screened for indels and left-right patterning defects and raised to adulthood. F2 homozygous mutant embryos obtained from subsequent generations will be analyzed for asymmetric expression of *dand5* in KV by in situ hybridization. Additionally, the subcellular localization of *dand5* RNA will be investigated by CRISPR-Cas13-GFP mediated live reporter tracking. Identifying its 3'-UTR elements will uncover how *dand5* is regulated and assessing the subcellular localization will reveal sites of regulation and their relevance in L-R patterning. These studies will bring us one step closer to elucidating the molecular mechanisms downstream of fluid flow that establish *dand5* asymmetry during L-R organogenesis.

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

Cellular Reconstitution of Drosophila Germ Granules

Irene Kim, Kira Mitchel, **Elizabeth Gavis**

Condensates of proteins and RNA known as germ granules are essential to species throughout the animal kingdom to sequester and target mRNA to the future germline. The germ granules of Drosophila exhibit a distinct spatial organization in which the RNA self-assemble into clusters with other transcripts from the same gene. However, the properties of the RNA that direct this homotypic assembly of RNA have not yet been identified. Furthermore, conventional RNA visualization and studies of endogenous RNAs in the Drosophila oocyte pose experimental limitations to the study of germ granule organization. Here, we present a cellular system to reconstitute Drosophila germ granules by heterologous expression of core germ granule proteins Oskar and Vasa and transfection of pgc and nos RNA. We observe that Oskar and Vasa exhibit high co-localization with each other in spherical aggregates, resembling their organization in the Drosophila oocyte. However, neither pgc nor nos RNA are recruited to reconstituted condensates. We plan to optimize the RNA transfection protocol to facilitate proper RNA localization with Oskar-dependent condensates and organization of RNA into homotypic clusters. This minimalistic in vivo system would enable the study of the RNA sequence and protein requirements for homotypic RNA clustering. Investigating the RNA and protein requirements for homotypic clustering should reveal broader principles governing the assembly of RNA complexes, which can be applied to the many types of RNP granules present in various species.

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Isolating Suppressors of the Skp SurA Synthetic Lethal Double Deletion in *Escherichia coli*

Tommy Kim, **Thomas Silhavy**

Escherichia coli are Gram-negative bacteria, characterized by having two membranes, the inner membrane (IM) and outer membrane (OM), which surround the cell.[1] The IM and OM are separated by the periplasm, and in order for outer membrane proteins (OMPs) to reach the outer membrane, these proteins must be transported from the cytoplasm, past the inner membrane, and through the periplasm.[1] OMPs are beta barrel proteins that are incorporated into the OM with the help of the Bam complex, and while moving through the periplasm, various chaperone proteins ensure that OMPs reach their destinations and fold properly.[1] The three chaperone proteins of interest in the following study are SurA, Skp, and DegP. Previous research has shown that SurA and the Skp/DegP pair have the same role in OMP chaperoning, with SurA having higher priority.[2] Previous research has also shown that while the ΔSurA ΔDegP double mutant can survive at low temperatures (at most 23°C), the ΔSurA ΔSkp double mutant cannot survive in any environment.[3] Suppressor strains of the ΔSurA ΔDegP double mutant has been used to better understand the σE stress response that *E. coli* experience when OMPs are compromised; however, experiments involving isolating suppressors using the lethal ΔSurA ΔSkp double mutant have been limited.[4] This study attempted to overcome the obstacle posed by the ΔSkp ΔSurA double mutant by first isolating a strain of *E. coli* containing the ΔSkp and ΔSurA deletions and a weak suppressor that allowed the strain to grow only on minimal media. A subsequent selection on rich media led to the development of strains with stronger suppressor mutations. Two strong suppressor candidates were also isolated during the initial synthesis of the double mutant strain by plating the double mutant directly onto rich media. After strain characterization through growth curves, efficiency of plating assays, and Western blots, the three most promising suppressor candidates were sent for whole genome sequencing in the hopes that these strains will contain mutations that merit further exploration.

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Development of a high-resolution, quantitative approach that links 3D chromatin conformation with transcriptional activity and regulatory interactions

Sarah Kuo, Michal Levo, Sergey Ryabichko, **Thomas Gregor**

Enhancer-promoter interactions drive gene regulation by affecting transcription and subsequent protein production. Previous research has found various links between enhancers and promoters which regulate transcription, and these interactions can range from spanning a few hundred base pairs to hundreds of kilobases in the genome. Additionally, further research has demonstrated that, within the eve locus, measured distances between any enhancer and the eve promoter in active loci are consistently less than 200nm, begging inquiry into the mechanisms and precise scales by which these distant segments of the genome coordinate with each other. By establishing a high-resolution computational approach in which fluorescence can be identified in images taken by confocal microscopy and subsequently used to both segment embryos to form a map of nuclei and demarcate tagged spots in the genome, we are able measure the physical distances between enhancers and promoters in single molecules of DNA. Using this robust quantitative method to measure three-dimensional chromatin interactions, as well as further identifying the presence of mRNA in nuclei to assign transcriptional activity status to genes using similar methods, we are able to better understand the physical scale of these enhancer-promoter interactions, as well as if they change with respect to domain and transcriptional activity. Application of this tool to control samples demonstrates the precision and robustness of the analysis pipeline, while application to wildtype and mutant embryos, for example to further investigate regulatory interactions in the eve locus, reveals enhancer-promoter proximity and dynamic chromatin conformation, dependent on both domain and transcriptional activity.

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

Capturing the dynamics of dolphin mobility

Sophie Leheny, Scott Wolf, Grace McKenzie-Smith, Marcelo Magnasco, **Joshua Shaevitz**

Dolphins are highly intelligent animals and participate in complex social interactions, such as name-based communication. It is thought that the ability of dolphins to maintain highly coordinated swimming in groups is key to the development of these social traits. Indeed, among the many forms of social communication dolphins engage in are small, controlled physical touches while swimming together. In the wild, dolphins display these motor skills in high turbidity environments, overcoming massive shear forces to maintain tight control of their motion. Forming a better understanding of these fine grained motions and other sensory cues, such as vocalization, will give valuable insight into dolphin dynamics and social function. In order to study these social behaviors, we use videos captured from sets of paired GoPro cameras attached directly to the bow of a boat. Dolphins exhibit both social behaviors and individual locomotion during 'bow riding,' where they gather and interact in the unique hydrodynamic environment created by the motion of the boat. We localized each body part in a 7-point skeleton in 2D using SLEAP, a machine learning software that generates predictions of animal location and limb position across videos with numerous animals. Following 2D localization, we reconstructed the skeleton in 3D using stereovision, a method of computing depth from two adjacent images. The goal of this image analysis is to better characterize dolphin motor skills and their use in social dynamics in the wild.

Stochastic modeling of sRNA-mRNA sequestration and reduction of translational burst noise in *Vibrio* quorum-sensing networks

Meryl Liu, Jacob Halatek, **Ned Wingreen**

Interactions between biomolecules in DNA replication, transcription, and translation possess inherent stochasticity, which leads to intrinsic noise in gene expression (Elowitz et al., 2002). Organisms deploy various methods for regulating this noise to maintain precise RNA and protein levels. In *Vibrio* quorum-sensing networks, small, regulatory RNAs (sRNAs) called Qrr (Quorum regulatory RNA) regulate protein production by a sequestration mechanism in which *luxO* mRNA and a Qrr form a complex, sequestering the *luxO* mRNA ribosome-binding site and thus repressing translation (Feng et al., 2015). Previous mathematical models indicate that accelerated catalytic degradation of mRNAs by sRNAs can reduce protein noise in both prokaryotes (Levine et al., 2007, Mehta et al., 2008) and eukaryotes (Schmiedel et al., 2015). However, those models do not consider how the inhibition of translation by mRNA sequestration affects intrinsic noise in protein expression. Here, we extend existing models to address the sRNA-mRNA sequestration mechanism in bacterial regulatory networks. Like mRNA degradation, reversible sequestration of mRNA shortens the free-mRNA correlation time, reducing variation in resulting levels of translated protein, but without degrading the mRNA. By simulating this reaction network using the Gillespie stochastic simulation algorithm (SSA), we show that sequestration can dramatically reduce translational burst noise. At high sRNA levels, protein noise is reduced to the Poisson limit. We also demonstrate the existence of multiple distinct regimes of noise reduction depending on model parameters, revealing that protein noise reduction by mRNA sequestration is much more complex than previously thought. Our findings open avenues for further exploration into the feasibility of noise reduction by mRNA sequestration, with implications for microRNA-based noise regulation in eukaryotes.
This research was made possible by the generous support of the Lewis-Sigler Institute for Integrative Genomics.

Dysregulation of Collagen Fibrillogenesis with Mutations in Fibronectin

Faith Meitl, Benjamin Lovett, **Jean Schwarzbauer**

Proper formation of the extracellular matrix (ECM) is crucial for the development of tissues and cell function. Fibronectin (FN) is an important component of the ECM that is assembled by cells into a fibrillar matrix that allows for the deposition of many other ECM components. Assembly of the abundant structural protein, type I collagen (COL I), depends on the FN matrix and provides strength to tissues and bones. Proteolytic cleavage of N- and C-terminal propeptides from the procollagen precursor molecule promotes its assembly. Formation of both FN and COL I matrices requires the glycosaminoglycan heparan sulfate (HS) which binds to FN at the HepII domain, primarily at the 13th type III module (III_{13}). Loss of HS and mutations in III_{13} have been associated with multiple diseases, including glomerulopathy with FN deposits and hereditary multiple exostoses. Previous work has generated two cell lines of mutant fibroblasts with heterozygous loss of the FN III_{13} domain (FN $III_{13}^{-/+}$ cells). In this study, I am analyzing FN and COL I fibrillogenesis by FN $III_{13}^{-/+}$ and wildtype fibroblasts using immunofluorescence images of COL I matrix, immunoblot analyses of insoluble matrix, and whole cell lysates. Immunoblot data of cell lysates and conditioned media show that wildtype and mutant cells produce and secrete similar amounts of procollagen and COL I. Preliminary data also indicates differences in procollagen cleavage between mutant and wild type cells. Future experiments will determine whether partial loss of FN III_{13} leads to differences in FN and COL I matrix formation and procollagen cleavage. This work will provide important insights into the mechanisms by which alterations to the FN III_{13} domain may result in diseases.

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Developing a Single-nucleus RNA-sequencing protocol for C. elegans

Maria Morales-Salgado, **Coleen Murphy**

Cell identification and characterization has led to several scientific discoveries and a comprehensive understanding of many types of cells across several organisms. The well-developed Single-cell RNA sequencing (scRNA-seq) technique has been central in achieving such identification of cells and the development of the human cell atlas. For Caenorhabditis elegans, scRNA-seq was used to identify neuron-enriched genes. However, single-cell seq protocols in C. elegans isolate different neuron classes with varying efficiency, and RNA-enriched neurites are often lost during cell preparation. With this goal in mind, this research focuses on creating a single nucleus RNA-seq protocol to acurately performs gene expression analysis across all C. elegans neurons in response to various experimental treatments or mutant conditions. Fluorescence-activated Cell Sorting (FACS) aided this process by differentiating the nuclei from cell debris produce by the lysed worms. To identify the nuclei specific to neurons, a green fluorescent protein worm strain was created which tagged all of the neurons of the worms. To get the highest number of fluorescent worms for the FACS machine, the strain was fully integrated such that all of the worms had neuron fluorescence. After passing the sample through the FACS machine, each individual cell-type and transcriptional state classification would be using 10X Genomics barcode labeling and sequencing.

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

Regulation of Peroxisome Morphology to Inhibit Antiviral Immune Signaling during HCMV Infection

Cody T. Mui, William A. Hofstadter, and **Ileana M. Cristea**

Subcellular organelles are dynamic regulators of cellular functions through constantly reorganizing their composition and morphology in response to cellular cues. Viruses utilize the dynamic nature of organelles to repurpose them for viral replication. This process is exemplified by human cytomegalovirus (HCMV), a virus that represents a significant global health burden particularly for infants and immunocompromised individuals. During infection, HCMV separates peroxisomes into distinct fragmented and enlarged populations. Concomitant with this polarization of peroxisomes is the inhibition of peroxisomal MAVS-induced antiviral immune signaling. However, the role and relevance of peroxisome morphology on peroxisome-mediated antiviral response, as well as the mechanism behind the polarization of peroxisome populations, remain unclear. Here, we aim to elucidate the proviral role of fragmented and enlarged peroxisomes on the inhibition of immune signaling using proteomic and reporter assays. These studies will develop a clearer understanding of the relationship between peroxisome form and function during viral infection, as well as mechanisms driving disease states involving peroxisome morphology.

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

Investigating IFI16 Immune Signaling and Phase Separation During HSV-1 Infection

Corazón Núñez, Dawei Liu, **Ileana Cristea**

The detection of pathogenic DNA by host sensors is a key step in activating host immune responses against microbial infection. Interferon-inducible protein 16 (IFI16) is a nuclear DNA sensor responsible for recognizing foreign DNA, such as the genomes of nuclear-replicating herpesviruses. Subsequently, IFI16 activates intrinsic innate immune responses via the induction of antiviral cytokines and the suppression of virus gene expression. In the context of herpes-simplex virus type I (HSV-1) infection, Cristea and coworkers have previously shown that the antiviral function of IFI16 is dependent on its ability to oligomerize upon sensing viral DNA. More recently, it has been established that liquid-liquid phase separation (LLPS) is also required for IFI16 immune signaling. However, the relationship between oligomerization and LLPS is not well understood, nor is it clear how IFI16 transmits the immune signal downstream to initiate cytokine induction. To examine how the ability of IFI16 to both oligomerize and phase separate mediates its immune signaling, we combined gain-of-function and loss-of-function mutants for each phenotype in the same IFI16 construct. Preliminary results using confocal microscopy demonstrate that both IFI16 oligomerization and LLPS are required for IFI16 organization into a fibrous network. Therefore, we predict that both phenotypes are also required for IFI16 to induce cytokine expression and inhibit viral replication. We hypothesize that IFI16 oligomerization and phase separation work in a cooperative manner to promote IFI16 immune signaling, rather than acting through independent mechanisms. Additionally, using proximity-labeling paired with mass spectrometry (PL-MS), we will identify downstream IFI16 interactors that induce immune signaling. This work will provide mechanistic insight into a critical intrinsic innate immune pathway during viral infection.

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

Light-Inducible Signaling Control in mESCs

Dev Patel, Evan Underhill, **Jared Toettcher**

Recent 3D organoid systems replicate late-gastrulation mammalian development, permitting an investigation of the signaling requirements of embryogenesis while avoiding the experimental constraints of the post-implantation embryo. These structures, called gastruloids, self-organize from aggregates of mouse embryonic stem cells (mESCs) into 3D structures with well-defined anteroposterior axes, allowing us to investigate the role of distinct signaling pathways in driving patterning and morphogenesis. We have observed, for example, that global platelet-derived growth factor (PDGF) addition leads to expansion of the anterior tissue and increased cell proliferation throughout the gastruloid. Conversely, fibroblast growth factor receptor (FGFR) inhibition significantly reduces axial elongation. While these pathways clearly have an important role during gastruloid development, the effect of local ligand secretion on cell differentiation and growth has not been studied. Using the blue-light dependent transactivator GAVPO, we hypothesize that locally inducing expression of growth factors such as FGFs and PDGF will drive local cell fate specification or directed morphogenesis in gastruloids. By varying the dosage and timing of this blue-light and observing transcriptional/morphological outputs, we hope to better understand the mechanism by which multicellular structures interpret and act upon morphogen gradients.

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

Quantifying Protein Turnover in *Drosophila Melanogaster* Embryos

Aleigha K. Reynolds, Edward Cruz, **Martin Wühr**

During embryogenesis, a single fertilised egg gives rise to thousands of cell types with distinct proteomes that are critical for establishing lineage specification and organogenesis. Thus, the regulation of protein concentration through post-transcriptional control and protein turnover is crucial for embryonic development. However, the role of protein turnover in setting protein abundances has largely been disputed and no present developmental study has directly measured protein turnover. Peshkin and Wühr *et al.* indirectly infer that protein degradation plays little to no role in controlling protein levels in vertebrates through mass action kinetics; however, Kronja *et al.* showed that the simultaneous degradation and synthesis of specific proteins in *Drosophila melanogaster* is concealed by protein levels which appear constant. Here, protein turnover is measured in *D. melanogaster* embryos using a stable isotope tracing strategy with $H_2{}^{18}O$ and multiplexed proteomics. Embryos are incubated on $H_2{}^{18}O$, heat-fixed at different hour intervals of development and a proteomic time series is generated using mass spectrometry. This method allows us to determine the distinct protein groups which are regulated primarily through protein turnover. Altogether, this investigation aims to provide new essential regulatory data and determine how protein abundances are controlled to facilitate their biological functions during body plan formation.

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

Dynamic Programming Improves Computational Identification of Tissue Layers

Rishabh Rout, Cong Ma, **Ben Raphael**

Spatial transcriptomics (ST) measures the location and genetic expression of cells on a 2D slice of tissue. This technique provides information regarding locational gene expression with unprecedented precision. Current models used for spatial transcriptomics either propose that gene expression has continuous variation across a tissue sample, or that a sample can be split into distinct cell types with homogenous gene expression. The Belayer model, however, relies on the idea that a tissue sample can be split into layers, where each layer may have distinct gene expression from neighboring layers while having continuous expression within. We aim to improve the accuracy of the model's layer creation by allowing it to introduce multiple segments into the layers, via dynamic programming. In fact, dynamic programming improves the efficiency of the model by computing the solutions to sub-problems and combining them to create precise layers dividing the ST sample. Such an improvement promotes a more biologically precise method of computationally representing variation in gene expression. Our results have potential applications in simulating tissue samples that contain normal and tumor cells.

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

Analyzing regulation of microtubule dynamics utilizing region specific fragments of spindle protein CKAP2

Michael Ruttlen Jr, Collin McManus, **Sabine Petry**

Regulation of microtubule (MT) dynamics during spindle assembly is required for precise chromosome segregation during cell division. Microtubule associated proteins (MAPs) are important proteins that bind to MTs and regulate their dynamics, including growth and shrinkage rates, as well as nucleation profile.CKAP2 operates like a microtubule polymerase, which has been characterized as a potent MT nucleation factor. It has been recognized to increase nucleation frequency, affect MT growth rate, and decrease depolymerization rates. Furthermore, it is generally upregulated in tumor cell lines as it is a p53 tumor suppressor target gene. CKAP2 does not yet have an identifiable mechanism that defines its regulation of MT dynamics. For this reason, it is important to analyze the mechanisms responsible for its activity and its effect on MTs. CDK1-cyclinB1 is an identifiable kinase that affects the activity of CKAP2 through phosphorylation C-terminal residues. Further, CKAP2 contains intrinsically disordered domains near the N-terminus that may contribute to its ability to phase-separate along MTs. Given that these two mechanisms are essential for CKAP2's interaction with MTs, We hypothesize that both phosphorylation and phase separation ability are important activities in how CKAP2 functions as a potent nucleation factor. We will investigate multiple constructs of CKAP2, isolating various amino acid sequences and testing for associated MT dynamics. This research will provide a better functional understanding of CKAP2 as a MT polymerase and characterize its activity with MTs.

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

Identification and Characterization of Host Factors Involved in Hepatitis E Virus Uptake

Olivia Sakaguchi, Michael Schwoerer, **Alexander Ploss**

Hepatitis E virus (HEV) is an etiological agent of viral hepatitis. Despite its significant burden on global health, much remains to be understood about its replication cycle, in particular the mechanism of viral entry into host cells. Although a few host molecules have been implicated in the HEV uptake process, the cellular receptor for HEV has yet to be identified. Characterizing the HEV receptor is essential for a number of drug development strategies targeting the cellular receptor. Entry is suspected to be mediated by a transmembrane receptor that is both necessary and sufficient for viral capsid internalization. To identify the receptor, I propose the use of partially tagged virus-like particles (VLPs) that will be used for proximity labeling in HEV permissive cells and subsequent identification of candidate proteins by mass-spectrometry. Preliminary results confirm that $ORF2_{112\text{-}608}$ is sufficient to generate VLPs in a baculovirus expression system, an essential step to conduct receptor labelling experiments. Propagation of HEV VLPs will aid efforts to identify the HEV receptor and clarify entry mechanisms.

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Effects of ERK Signaling on Protein Expression in *Drosophila* Embryos

Milan Schillaci, Audrey Zhu, Liu Yang, **Stanislav Shvartsman**

The ERK signaling pathway plays several important roles in the development of many different organisms, including humans and *Drosophila melanogaster*. Disruptions in this pathway have been linked to defects such as Fragile X syndrome, which is the most common cause of inherited intellectual disability. While we do know that several different genes are activated and repressed downstream of ERK, it is still not known exactly how many genes are connected to this pathway and where all of their associated proteins are expressed at different stages of development. By using mass spectrometry to compare the protein levels of *Drosophila* embryos with and without ERK activation, we hope to determine which proteins are affected by disruptions to this pathway. We also demonstrate that it is possible to create light-sheet movies that track the expression of these proteins through the many nuclear cycles that occur during embryonic development. These results will provide greater insight into the many effects of the ERK signaling pathway and the proteins that may contribute to developmental defects.

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Examining the role of envelope protein mutations in promoting the dengue viral life cycle

Emily Schoeman, **Alexander Ploss**

Dengue viruses (DENVs), members of the Flaviviridae family, are a group of single stranded, positive sense RNA viruses transmitted to humans primarily through mosquito vectors. DENVs present a significant global health burden, infecting an estimated 390 million people each year, yet vaccination and treatment efforts have been hindered by a lack of knowledge of the DENV life cycle. To gain insights into crucial host-virus interactions governing the DENV cycle, we adapted DENV strains by serial passaging to robustly infect human keratinocyte cells which are minimally permissive to DENV infection. We chose keratinocyte cells since this is one of the first cell types the virus encounters following transmission by a mosquito bite. Through whole genome sequencing, common mutations were identified in the adapted strains that conferred a significant fitness advantage in cell culture, including two mutations in amino acid 327 (E327K, E327T) of the envelope (E) protein. To build upon this work, the current study aims to investigate the impact of these mutations on the ability of the virus to carry out its infectious cycle. Firstly, circular polymerase extension reaction was used to introduce the E327K and E327T mutations into an infectious clone of the parental virus and in vitro growth curve analysis was conducted in both hepatic and keratinocyte cells to compare mutant and wildtype viral fitness. To pinpoint which viral life cycle steps these mutations affect, extracellular and intracellular virion quantities were compared by viral titration and in vitro transcribed viral RNA was transfected to measure the genome replication rate. Further, a lipophilic fluorescent dye was utilized to visualize the impact of the E protein mutations on viral entry through flow cytometry. The results of these assays suggest a role of the region encompassing the E protein mutation in promoting viral egress; proximity labelling techniques using a short peptide containing this region as bait will be used to identify and ultimately characterize candidate interactors in different cell lines. Taken together, these results will provide mechanistic evidence for dengue viral egress and potentially uncover host cell proteins involved in the DENV life cycle, a critical step towards therapeutic development.

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Characterizing the Immunosuppressive Effect of Tumor-Derived Retinoic Acid on Dendritic Cells

Larry Shue, Cao Fang, **Yibin Kang**

Cancer cells are often able to evade immune surveillance, facilitating tumor progression and creating a barrier for developing effective immunotherapy. The secretion of retinoic acid (RA) by tumor cells is known to suppress immune responses by promoting the formation of an immunosuppressive tumor microenvironment (TME). Recent studies have demonstrated that expression levels of alcohol dehydrogenase family1, member A2 (ALDH1a2), which converts retinal to RA, is upregulated in tumor-associated dendritic cells (DCs) in response to RA signaling, which promotes regulatory T cell differentiation and immune tolerance. Considering that most human cancer cell lines express a similar ALDH1a3 isoform, it is hypothesized that cancer cells secrete RA to induce an immunosuppressive TME by upregulating ALDH1a2 expression in tumor-associated DCs, shifting them towards a more regulatory phenotype. Co-cultures of B16-F10 melanoma cells with or without ALDH1a3 overexpression and murine bone marrow cells revealed no change in the proportion of DC differentiation, although DC ALDH1a2 expression increased when co-cultured with B16-1a3 cells. Interestingly, the presence of any tumor cells in the co-culture significantly increased neutrophil differentiation. In an *in vivo* experiment, B16-MCS or B16-1a3 cells were injected subcutaneously into the flanks of B6 mice, and ALDH1a3 appeared to slow tumor growth, hinting at an immune-unrelated effect of ALDH. Further characterization of the co-cultured and tumor-associated DCs will uncover whether they are phenotypically affected by the tumor-derived RA signaling pathway, potentially revealing a mechanism in which cancer cells accomplish immune escape.

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Investigating the Biochemical Interaction between Eg5 and TPX2 and Its Role in Branching Microtubule Nucleation

Haley Tran, Venecia Valdez, **Sabine Petry**

Cell division is essential for life. During mitosis, a bipolar spindle is built from microtubule filaments to align and segregate chromosomes. Branching microtubule nucleation is a unique spindle assembly pathway and is characterized by microtubule nucleation initiated along the sides of pre-existing microtubules. Previous studies show that the spindle assembly factor TPX2 is required for branching microtubule nucleation in Xenopus laevis. During mitosis, TPX2 interacts with the plus-end directed kinesin motor, Eg5, to regulate spindle assembly and organization. However, it is unknown whether the Eg5-TPX2 interaction is specific to the branching pathway. Preliminary results and prior studies show that TPX2 lacking the Eg5-binding domain (TPX2ΔEg5) fails to bind microtubules in Xenopus laevis egg extract and cannot rescue branching microtubule nucleation when endogenous TPX2 is immunodepleted. However, in vitro, TPX2ΔEg5 is capable of binding microtubules. Here, I will investigate the molecular basis for the interaction between Eg5 and TPX2 in branching microtubule nucleation. To determine how Eg5 interacts with TPX2, I am utilizing site-directed mutagenesis and Gibson assembly to create TPX2 and Eg5 constructs containing systematic deletions, subdividing Eg5-binding domain of TPX2 into thirds and isolating the motor, stalk, and tail domains of Eg5. I will then perform pulldown assays to refine and elucidate the Eg5-binding site and TPX2-binding site in TPX2 and Eg5, respectively. These constructs will then be used for TIRF microscopy assays to test whether Eg5-TPX2 binding is necessary for branching microtubule nucleation in Xenopus laevis egg extract. Together, this research will demonstrate the role of Eg5-TPX2 interactions in branching microtubule nucleation during spindle assembly.

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Mechanical Stress Alters Nuclear Condensate Rheology

Michael Tsai, Jessica Zhao, **Clifford Brangwynne**

Liquid-liquid phase separation (LLPS) is an emerging biophysical mechanism for compartmentalizing nuclear functions, such as gene regulation in the form of biomolecular condensates. In particular, previous studies on the nucleus mechanics showed chromatin's influence on biomolecular phase separation inside the cell nucleus and condensate kinetics via largely mechanically unperturbed states. Furthermore, altered chromatin mechanics in bulk, global perturbation can affect nuclear architecture and rheology. To better interrogate the differential viscoelasticity at local chromatin environments under mechanical stress, we use engineered optogenetic condensates as passive microrhelogical probes in metastatic epithelial cells under confined cell migration. Our preliminary data suggests that confined migration restricts condensate motion at the posterior of the cell nucleus. Our results add to the library of known mechanical properties of chromatin and open up new questions on the spatial-temporal genome regulation implications of chromatin network's mechanics.

Purification of the Qb-SNARE Vti1

Alex Tsai, **Fred Hughson**

Eukaryotic cells use vesicles to transport materials into, out of, and within the cell. These intracellular trafficking events culminate with membrane fusion which is mediated by soluble *N*-ethylmaleimide-sensitive factor attachment protein receptors (SNAREs), Sec1/Munc18-like (SM) family proteins, Rab GTPases, and their effectors. Just as SNAREs catalyze the membrane fusion event, SM proteins catalyze the SNARE complex assembly in the first place. The HOPS complex is a multi-subunit tethering complex (MTC) that contains the SM protein Vps33 and regulates late endosomal trafficking. The four SNAREs associated with HOPS in the late endosomal pathway are the R-SNARE Nyv1, and the Qa-, b-, and c-SNAREs Vam3, Vti1, and Vam7, respectively. While Vps33 has been shown to initially bind Nyv1 and Vam3 in a template complex to catalyze full SNARE complex assembly, the mechanism by which Vti1 and Vam7 become associated with the partial SNARE complex is still unclear. On top of this, while HOPS is believed to eventually bind all four SNAREs in their complete complex, binding data for Vti1 is sparse. An understanding of where and how Vti1 binds as the SNARE complex is assembled would provide a more complete model of how HOPS facilitates full SNARE complex assembly in the late endosomal pathway. Purification of Vti1 is required for this investigation, and once purified, Vti1's orientation in context to HOPS will be studied via initial co-incubation with the HOPS Vps33, Nyv1, and Vam3 template complex and subsequent visualization via x-ray crystallography and electron microscopy.

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Development of a Mismatch Repair Assay using Prime Editing

Katya Williams, Jun Yan, **Britt Adamson**

Huntington's disease (HD) is a late-onset, fatal neurodegenerative disorder that results from an excess of CAG trinucleotide repeats in the *HTT* gene. Somatic expansion of this repeat region is inhibited by the loss of MutSβ, a DNA mismatch repair protein that recognizes insertion-deletion loops. Inhibiting MutSβ would provide a potential treatment for HD. However, no compounds are currently known to specifically inhibit MutSβ activity. A major challenge in identifying these compounds is creating an assay to measure MutSβ activity. Our lab aims to create a substrate-based reporter using prime editing, a CRISPR-based gene editing tool capable of introducing mismatch repair substrates into the genome of living cells. We will then measure the expression of the reporter in the presence of various compounds to evaluate their MutSβ-inhibiting potential. Using prime editing to activate the reporter gene provides our lab with a flexible, sensitive way to quantify MutSβ activity and thus identify potential strategies for HD treatments.

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Development of a Mismatch Repair Assay using Prime Editing Time-Dependent Telomerase Inhibition in Human Cytomegalovirus Infection

Karen Yang, Chloe Cavanaugh, and **Daniel Notterman**

Human cytomegalovirus (HCMV) is a common beta herpesvirus that causes lifelong infection. Although often asymptomatic in immunocompetent hosts, active HCMV infection in immunocompromised people and developing fetuses can lead to severe morbidity, and there is no cure or vaccination for HCMV. A previous study by Stråat et al. (2009) found that HCMV upregulates the activity of telomerase, the reverse transcriptase responsible for maintaining the protective DNA sequences located at the ends of chromosomes. Telomerase is implicated in stress, aging, and cancer, and preliminary data shows that telomerase inhibition is associated with significant reduction of HCMV viral replication (Cavanaugh and Notterman, unpublished results). The overarching goal of this project is to examine the relationship between telomerase activity and HCMV. First, to examine the importance of telomerase in the viral life cycle, I inhibited telomerase activity at strategic timepoints within the viral life cycle with pharmaceutical telomerase inhibitor MST-312. Preliminary data shows that telomerase inhibition at the immediate early stage of the viral life cycle results in the lowest levels of viral titer, which suggests that host telomerase is most important at the immediate early stage. To confirm these results, I will repeat the experiment with another telomerase inhibitor BIBR1532 that utilizes a different mechanism than MST-312. To establish that the effect of telomerase inhibition is not an off-target effect, I will also suppress telomerase expression with a Tet-inducible shRNA construct and determine the effect on viral replication level. This research will contribute to understanding the role of telomerase in HCMV infection and potentially lead to new therapeutic options.

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Characterizing the Heterogeneity of Quorum Sensing Regulation in *Vibrio cholerae* Biofilms

Michelle Yoon, Boyang Qin, **Bonnie Bassler**

Coordination of gene expression is required for complex bacterial processes such as virulence, host infection, and biofilm formation. Such coordination is orchestrated via a cell-to-cell communication process called quorum sensing (QS). In the model bacterium and global pathogen *Vibrio cholerae,* the accumulation of autoinducers in the local environment facilitates the transition between the sessile biofilm lifestyle and motile planktonic lifestyle. While previous studies on other bacterial species suggest that biofilms are heterogeneous and contain cells that are physiologically distinct, gene expression heterogeneity in *V. cholerae* biofilms has yet to be identified. Filling this gap brings new understanding of how biofilms exhibit increased tolerance to antibiotics, as well as structural resilience in fluctuating environmental conditions. We investigate the heterogeneous gene expression in *V. cholerae* biofilms by examining spatial patterns of the QS master regulators HapR and AphA using advanced microscopy techniques, including confocal and lightsheet microscopy, that allow us to perform spatiotemporal analyses of living, growing biofilms at single cell resolution. Furthermore, we integrate machine learning algorithms to (i) identify cell position and gene expression levels at single cell resolution and to (ii) classify heterogeneous populations of cells and the divergence of cell fates. Our study will yield an understanding of how QS mechanisms regulate gene expression patterns, which would allow us to manipulate such patterns for future therapeutic interventions.

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Plant Colonization

Dinie Zheng, **Jonathan M. Conway**

Plants have a microbiome composed of a variety of microorganisms that interact closely with one another. Interactions between plants and their associated microbiome impacts plant phenotype. Interestingly, the microbial diversity within the plant microbiome is less than that of the surrounding soil, but the genetic basis that determines microbiome composition is still unknown. Prior studies determined that the innate plant immune system is one major factor influencing the composition of the plant microbiome and the ability to suppress the plant immune system is important for plant colonization. However, the genetic mechanisms that confer immunosuppression are also unknown. Here, we will investigate the genetic basis for plant colonization and immunosuppression using random bar code transposon-site sequencing (RB-TnSeq), which will generate mutant libraries of plant-associated bacteria and track microbial fitness. These mutant libraries will be screened for genes that impact plant colonization and immunosuppression. The mutant library for *Variovorax* CL14, a bacterial species known to be a strong plant colonizer, will be subjected to a competitive colonization screen to identify genes that influence plant colonization, and mutant libraries for bacteria that suppress the plant immune system will be subjected to a GUS histochemical assay to screen for genes related to immunosuppression. Identification of these genes will provide a better understanding of the mechanisms utilized by microbes to affect their plant hosts, enabling bioengineering of these mechanisms to improve agriculture and plant health.

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Optimizing metabolic fluxes via "sticky" enzymes

Jello Zhou, Alejandro Martinez-Calvo, Yaojun Zhang, **Ned Wingreen**

Phase separation represents a crucial and ubiquitous mechanism in biology, its most prominent example being intracellular organization of membraneless organelles. Previous work has shown that enzyme clustering accelerates and improves the efficiency of metabolic pathways. For instance, efficiency-maximizing enzyme ratios have been found in bacteria and purinosomes in humans. However, the underlying mechanisms of enzyme clustering and the acceleration of intermediate processing remain poorly understood. Here, we present a theoretical model to show that the spontaneous clustering of enzymes via phase separation accelerates the process of intermediates in a two-step metabolic pathway. To this end, we consider a mixture of four different enzymes, each two of them responsible for processing a substrate and an intermediate, employing the Flory-Huggins and Model B formalisms. We perform full numerical simulations to quantitatively analyze the pattern formation of enzymes, substrate, and intermediate, and the efficiency of the two-step metabolic pathway when enzyme clustering takes place.

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Identifying dynamic m6A sites and the targets of RNA-binding proteins via APOBEC-mediated deamination

Yaakov Zinberg, **Ralph Kleiner**

m^6A is the most abundant modification in the eukaryotic transcriptome and is involved in many aspects of RNA metabolism. Previous studies indicate m^6A is altered under stress, suggesting that m^6A plays an important role in the cellular stress response, yet certain stress-related m^6A sites have only been identified using immunoprecipitation-based methods, which require an antibody and lack high resolution. The uncertainty surrounding the function of stress-induced m^6A may arise from the lack of precision and efficiency inherent to these antibody-based methods. DART-seq uses a fusion protein of the m^6A-binding YTH domain with APOBEC, a cytidine deaminase, to induce mutations near m^6A sites, but it lacks the temporal control needed to precisely identify dynamic m^6A sites. This study will develop a new method of identifying dynamic m^6A sites by coordinating stress induction with APOBEC-mediated deamination. The method will be used to identify and characterize dynamic m^6A in cells undergoing oxidative stress, a state associated with many human diseases such as cancer and Alzheimer disease. Completion of this study will improve our understanding of how m^6A modulates mRNA fate under oxidative stress and introduce a method that can be used to study m^6A dynamics under other physiological conditions.

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