Research Project Title: Green fluorescent protein (GFP) and GFP binding protein (GBP) tagging in Aspergillus nidulans

Student Presenter: Leymaan Abdurehman

Faculty Mentor: Stephen Osmani

Faculty Mentor Department: Molecular Genetics

Research Abstract: Green Fluorescent Protein (GFP) tagging is commonly used to identify locations of proteins and is an important step towards understanding a proteinâ€™s function. Live cell microscopy after GFP-tagging is as an excellent way to study proteins of interest and has been widely used in the model filamentous fungus Aspergillus nidulans. To further enhance experimental approaches after GFP-tagging, we have adapted the GFP-Binding protein (GBP), a protein that binds GFP with high affinity, for A. nidulans (Suresh et al., 2017). Tagging anchor proteins with GBP causes GFP-tagged proteins to be re-targeted to the location of the GBP-tagged anchor protein. Four DNA-cassettes were generated that, through fusion PCR, can be used to generate specific GBP-tagging constructs. Using the cassettes, two strains were generated expressing either SPA10-GBP or Tom20-GBP. SPA10 locates to the septa and Tom20 locates to the surface of mitochondria. Using live cell-microscopy, I was able to determine that SPA10-GBP successfully relocates GFP-tagged proteins to forming and mature septa. In the Tom20-GBP strain, GFP-tagged proteins located to the surface of the mitochondria. Notably the higher abundance Tom20-GBP anchor was able to attract more GFP-tagged protein than the SPA10-GBP anchor. Both the SPA10-GBP and Tom20-GBP strains generated can be used to further investigate the consequences of re-targeting other GFP-tagged proteins to ectopic locations in A. nidulans.

Research Project Title: Activation of 3-hydroxypropionate by addition of coenzyme A in Rhodobacter sphaeroides

Student Presenter: Sydney Alibeckoff

Faculty Mentor: Birgit Alber

Faculty Mentor Department: Microbiology

Research Abstract: Rhodobacter sphaeroides is a metabolically diverse bacterium that can grow with a variety of carbon sources, including the organic acid 3-hydroxypropionate. This molecule is used in the synthesis of industrial chemicals and polymers, and understanding how it is metabolized may have important applications. To be fully assimilated by R. sphaeroides, we hypothesize that 3-hydroxypropionate is activated via addition of a coenzyme A (CoA) thiol. The purpose of this study is to determine the enzyme responsible for converting 3-hydroxypropionate into 3-hydroxypropionyl-CoA. One class of enzymes that we hypothesize to be responsible for catalyzing this reaction is AMP-forming synthetases, which hydrolyze ATP to add a free CoA to the target molecule. 3-hydroxypropionate and CoA dependent hydrolysis of ATP was readily detectable in cell extract of cells grown with 3-hydroxypropionate as the sole carbon source. To test if synthetases are responsible for the conversion of 3-hydroxypropionate to 3-hydroxypropionyl-CoA, mutant analysis was performed. Two synthetase genes were bioinformatically identified in the genome of R. sphaeroides encoding a putative acetyl-CoA synthetase (acs) and propionyl-CoA synthetase (pcst). Both genes were inactivated by homologous recombination, exchanging the wild type gene with an in frame deleted copy. The resulting mutant strains were RsΔacs and RsΔpcst; RsΔacs was then used to generate the double mutant RsΔacsΔpcst. Cell extracts of RsΔpcst and RsΔacsΔpcst mutants have reduced synthetase activity on 3-hydroxypropionate when compared to wild type, which supports the hypothesis that pcst encodes a synthetase that can act on 3-hydroxypropionate. Initial growth experiments of the mutant strains with 3-hydroxypropionate indicate that while Pcs may play a role in 3-hydroxypropionate assimilation, the loss of Pcs still allows growth on 3-hydroxypropionate. We can conclude from mutant analysis that the loss of the pcst gene correlates to a decrease of synthetase activity with 3-hydroxypropionate. However, the preliminary data from growth experiments and enzyme assays lead us to believe that synthetases are not solely responsible for the conversion of 3-hydroxypropionate to 3-hydroxypropionyl-CoA. Other classes of enzymes that may catalyze this reaction include transferases and kinases-transacetylases.
Research Project Title: Determining binding specificities of cell adhesion molecules from Drosophila and other related Dipterans

Student Presenter: Leah Anderson

Faculty Mentor: Mark Seeger

Faculty Mentor Department: Molecular Genetics

Research Abstract: Neurons of both vertebrates and invertebrates exhibit a complex set of cell-to-cell interactions during successful development of the nervous system. Cell adhesion molecules (CAMs) play an important role in mediating many of these specific and stereotyped cell-cell interactions. I am investigating the binding specificities of two CAMs of the immunoglobulin superfamily from Dipteran insects: Lachesin (Lac) and Amalgam (Ama). Ama arose as a duplication of Lac early in Dipteran evolution, and both proteins still share extensive sequence similarity. In Drosohila melanogaster, Lac is membrane-linked and homophilically binds itself. Ama, which is a secreted molecule, has both a homophilic binding property as well as the ability to heterophilically bind another CAM, the transmembrane protein Neurotactin (Nrt). Despite the high level of amino acid sequence similarity between Ama and Lac, the two proteins are unable to bind each other, and Lac does not display any interaction with Nrt. The goal of this project is to identify the precise domain(s) of Lac and Ama that produce these differences in binding specificity. To accomplish this, chimeric constructs of the three immunoglobulin-like domains of Ama and Lac from D. melanogaster have been created and cloned into a vector for regulated expression in Schneider 2 (S2) cell lines. The S2 cells are then to be used for aggregation assays, which will reveal binding patterns of the chimeric proteins. Preliminary aggregation assays have revealed that the first immunoglobulin domain is responsible for the homophilic binding specificity of both Lac and Ama. Further experiments to test the secreted version of the Ama/Lac chimeras will allow identification of the domains that contribute to Nrt binding and Nrt-mediated cell adhesion. Using this approach, a thorough model can be devised for the specific interactions of Lac, Ama, and Nrt in D. melanogaster. In addition to studying these protein interactions, I am utilizing bioinformatic databases to locate and subsequently clone out orthologs of Ama and Lac in other Dipteran species. Testing these clones in further aggregation assays will help develop a better understanding of how the unique binding properties of Ama and Lac have changed over evolutionary time.
Research Project Title: The structural fate of spliced tRNA introns: linear or circular

Student Presenter: Alicia Bao

Faculty Mentor: Anita Hopper

Faculty Mentor Department: Molecular Genetics

Research Abstract: Transfer ribonucleic acids (tRNAs) are abundant molecules, comprising ~15% of cellular RNAs. Although the major biological role for tRNAs is to bring amino acids to the ribosome during protein synthesis, they also play many secondary roles. Defects in pre-tRNA biogenesis and processing cause numerous disorders, from neurodegenerative diseases to cancer. In eukaryotes, a subset of tRNA-encoding genes contains non-coding introns that must be removed in post-transcriptional tRNA processing; in S. cerevisiae, these account for 20% of pre-tRNAs. Through an unbiased screen of the yeast genome, we identified two proteins required for tRNA intron turnover of tRNAIleUAU, the tRNA employed in our screen. We showed that the free tRNAIleUAU intron is first phosphorylated on the 5\' end by the tRNA ligase/kinase Rlg1, then degraded by the 5\' to 3\' exonuclease Xrn1. By expanding my analysis to the other intron-containing pre-tRNAs, I identified the possibility for three more mechanisms for intron degradation, as well as circularization of the tRNATrp intron in vivo. The circular form is seen on Northern blots as an aberrant band running below the linear intron. The aberrant form is resistant to digestion by Terminator Exonuclease (TEX), a 5\' to 3\' exonuclease, suggesting it has no available 5\' end. Additionally, when reverse transcription polymerase chain reaction (RT-PCR) is performed on the tRNATrp intron, reverse transcriptase loops around the template sequence over and over, transcribing a long cDNA strand containing multiple repeats of the intron sequence. During the PCR step, primers hybridize to multiple sites on the cDNA transcript, creating concatemeric DNA, which can be visualized on an agarose gel as a series of bands that are multiples of the length of the original intron. This data is novel, as it was previously believed that introns in S. cerevisiae only existed in linear forms. Interestingly, only the tRNATrp intron circularizes in wild type cells; however, circular introns can be formed in other tRNA families if Rlg1 is replaced with the bacterial ligase, RtCB. Together, these findings indicate the multiplicity and specificity of tRNA intron degradation pathways, and suggest additional novel pathways exist for the turnover of novel intron forms.
Research Project Title: Inhibition of micro-RNA 126 to prevent Kasabach-Merritt Phenomenon in endothelial cell tumors

Student Presenter: Emma Clark

Faculty Mentor: Gayle Gordillo

Faculty Mentor Department: Plastic Surgery

Research Abstract: Hemangioendothelioma (HE) is a type of endothelial cell tumor. Kasabach-Merritt Phenomenon (KMP) can develop in infants with HEs and has a mortality rate of 20%. Infants with KMP die from consumptive coagulopathy with sequestration of blood and platelets in the tumor. Treatments for HE include chemotherapy agents, such as vincristine, that have high risk side effects. The purpose of this work was to test whether microRNA-126 (miR126) prevents KMP through repression of mammalian Target of Rapamycin (mTOR). mTOR is a protein kinase that promotes angiogenesis, which increases susceptibility to KMP. p85α/PIK3R2 is a protein that represses mTOR. Hemangioendothelioma endothelial (EOMA) cells, a validated HE model in 126P/3 mice, have high levels of expression for miR126 and mTOR, which were measured using qPCR and western blots. EOMA cells were transfected with miR126 inhibitors or control inhibitors to measure, using qPCR and western blots, expression of p-85α and mTOR. Results indicated that inhibition of miR126 increased p85α expression, which decreased mTOR expression. To analyze miR126 inhibition in vivo, 126P/3 mice will be injected subcutaneously with EOMA cells and treated with either miR126 inhibitors or control inhibitors. Blood testing should indicate decreasing levels of hemoglobin, hematocrit, and platelets in the control inhibitor group, indicative of KMP, compared to the miR126 inhibitor. Overall, activation of mTOR is miR126 dependent because miR126 silences p-85α, resulting in mTOR activation. Inhibition of miR126 resulted in mTOR repression by p-85α, which should prevent KMP. By determining the effects of miR126, we will be closer to discovering a safe and effective treatment for KMP. This will improve lives and decrease the death rate due to KMP.
Research Project Title: Characterization of multiple novel aperture proteins in Arabidopsis thaliana

Student Presenter: Ayla Edwards

Faculty Mentor: Anna Dobritsa

Faculty Mentor Department: Molecular Genetics

Research Abstract: In many species, exine, the outer wall of pollen grains, is deposited non-uniformly on the pollen surface. The gaps left on the pollen surface by the absence of exine deposition are known as apertures. These apertures facilitate emergence of the pollen tubes, making them important for male fertility in some plants. The positioning of the apertures is highly conserved within species, yet it varies widely between species, making apertures an ideal model for studying how cells control formation of distinct extracellular domains. Very little is known about how the locations of apertures are specified and how apertures are formed. Dobritsa Lab’s current work involves identification and characterization of novel proteins in Arabidopsis required for aperture formation. My project focuses on two of the mutants recently found during a mutagenesis screen, both of which completely lack apertures. The candidate genes responsible for these mutations, called Strubbelig-Receptor Family 2 (SRF2) and Inaperturate Pollen 2 (INP2) were isolated, fused with YFP, and introduced into their respective mutants in Arabidopsis using Agrobacterium. I created the constructs and confirmed the identity of these genes by phenotypic rescue, and their expression and localization are currently being characterized using YFP. In addition, I am also testing the effects of inactivation of ELMOD-A, a close paralog of a third gene involved in aperture formation called Macaroon, by mutating ELMOD-A using CRISPR. Mutant plants have been obtained and are being crossed with macaroon, inp2, srf2, and other aperture protein mutants to create double mutants and further characterize how the proteins interact. Characterizing these new aperture proteins will help elucidate the molecular mechanisms involved in the formation of distinct cellular domains.
Research Project Title: Reb1 is a pioneer factor that dynamically regulates nucleosomal DNA accessibility

Student Presenter: Caroline Jipa

Faculty Mentor: Michael Poirier

Faculty Mentor Department: Physics

Research Abstract: For efficient storage and protection, DNA in cells is packaged into chromatin. Chromatin’s basic unit is the nucleosome, 147bp of DNA wrapped around the histone octamer. Nucleosomes play important roles in regulating gene expression; the DNA in a nucleosome is inaccessible to most transcription factors (TFs) and other DNA binding proteins. Additional suppression of transcription stems from higher-order nucleosome compaction. So how does transcription begin in these highly compacted regions of the genome? A class of TFs called pioneer factors possess the unique ability to access binding sites within heterochromatin to initiate transcription and affect cell differentiation. However how this is achieved remains unknown. In this study, we develop a model for how pioneer TFs target their sites in individual nucleosomes.

Reb1 is an essential TF from S. Cerevisiae that establishes correct nucleosome positioning at the 5’ ends of genes. Gel shift experiments show that, unlike other TFs, Reb1 has a similar affinity to DNA and to nucleosomes. Ensemble FRET experiments indicate Reb1 targets its binding site on unwrapped nucleosomes without evicting the octamer. Additionally, single-molecule fluorescence experiments show that despite similar affinities, Reb1 displays dramatically slower exchange kinetics to nucleosomes than to DNA. From these results, we propose that Reb1 can function as a pioneer factor because it has high affinity for nucleosomes. Together this data could suggest that long dwell times from entry-exit sites allows for recruitment of chromatin remodelers leading to correct nucleosome positioning. Future experiments to characterize binding include EM structural studies, footprinting and mutagenesis studies. This may provide models for other pioneer factors, opening the door to bioengineering of molecules to control gene expression and regenerative medicine.
Research Project Title: The role of Rho5 in the apoptotic response of budding yeast

Student Presenter: Jack Fioretti

Faculty Mentor: Hay-Oak Park

Faculty Mentor Department: Molecular Genetics

Research Abstract: Apoptosis is a process of programmed cell death to remove cells that are no longer needed during development or damaged cells from stressful conditions. I am interested in understanding how cells of budding yeast Saccharomyces cerevisiae respond to harmful levels of reactive oxygen species (ROS), a byproduct of the metabolism of oxygen, and activate the apoptotic response. This response is a defense mechanism to remove those cells with excessive damages in the genome, which could potentially lead to a host of subsequent genetic errors in organelles. I am in the process of corroborating previous findings in the lab that the Rho5 GTPase is necessary for cell death upon damage by oxidants or heat stress in budding yeast. By subjecting wild type (WT) and rho5 deletion mutant strains to identical heat shock treatments, I will use a spot test assay to quantify cell survival in response to these stresses. I expect to observe that the WT cells exposed to the heat treatment will undergo a greater rate of apoptosis, as if they had been subjected to hydrogen peroxide, while the rho5 deletion mutant will be more resistant to the stress. After confirming the labâ€™s previous findings regarding these strains, I will seek to gain a deeper understanding of Rho5 GTPaseâ€™s molecular role in apoptotic response by identifying potential targets of Rho5. Specifically, I will test Cwp1/2 (cell wall proteins that contribute to propionic acid and low pH resistance), which were identified from previous genome-wide studies in the lab, by a BiFC (bimolecular fluorescence complementation) assay. The results of this analysis should provide insight into how Rho5 might interact with these proteins and contribute to apoptotic cell death. These findings may help us to understand how small GTPases might regulate apoptosis in animal or plant cells in response to stress or during fungal pathogenic infection.
Research Project Title: Functional analysis of the telomere (TLO) expanded gene family in Candida albicans

Student Presenter: Griffin Kinney

Faculty Mentor: Matthew Anderson

Faculty Mentor Department: Microbiology

Research Abstract: Candida albicans is a common commensal of the human gastrointestinal (GI) tract, however it is the cause of 50% of fungal infections, and the 4th most common hospital acquired bloodstream infection. Significantly, bloodstream infections result in mortality rates approaching 50%. C. albicans virulence is due, in part, to its ability to adapt to altered environments and the host immune system. Genes that confer a selective advantage to these unique environmental challenges faced by the organism are often found at the ends of the chromosome. Expansion of gene families through increases in gene copy numbers have been associated with virulence in C. albicans. The telomere associated (TLO) genes represent the most dramatic expanded gene family. The TLO gene family consists of 14 genes separated into 3 clades (α, β, and γ). TLO’s share approximately 97% sequence similarity, all encode a Med2 domain which has been shown to interact with transcription regulation. To analyze individual TLO gene functions, a drug-ON misexpression system was used where doxycycline activated expression. Misexpression strains for 12 of the 14 genes were constructed by replacing the promoter of one allele of a TLO gene with this inducible promoter. Strain phenotypes were assessed between induced and uninduced states, in both the parental strain and strains with specific TLO genes under misexpression. These misexpression strains were assayed to test the role of the TLO’s in a diverse range of environmental pressures. All TLO genes showed involvement in multiple phenotypes, as well phenotypes were shown to be controlled by multiple TLO’s. However, no phenotype was controlled by all TLO genes, indicating the development of either novel or delegated functions in relationship to the ancestral gene from which expansion acted on. TLO genes were shown to be involved in both simple phenotypes such as cell aggregation and complex phenotypes such as Galleria mellonella virulence. Sequence analysis of the TLOs showed multiple sequence locations that correlated with specific observed phenotypes. These results suggest evolutionary pressures have acted on the TLO gene family to cause regulatory diversification. We purpose this diversification of phenotypic regulation advances the pathogenic and commensal success of Candida albicans.
Research Project Title: Using molecular dynamics to characterize mutants in the connector region of Rho

Student Presenter: Max Gilliland

Faculty Mentor: Irina Artsimovitch

Faculty Mentor Department: Microbiology

Research Abstract: Transcription termination factors are necessary to silence synthesis of aberrant RNAs. Bacterial Rho protein is an archetype of factor-dependent termination. In Escherichia coli, Rho inhibits expression of anti-sense, corrupted, and horizontally-acquired RNA messages. E. coli Rho is a hexamer made up of 419 amino acids per subunit. Rho is an ATP-dependent, RecA-family hexameric helicase composed of an N-terminal RNA-binding and a C-terminal ATPase/helicase domains separated by a flexible 30-residue long connector region. A key step in the Rho mechanism is a switch from an open, RNA-loading state into a closed, translocation-competent state in which the RNA is captured inside the inner pore; this switch is activated by Rho binding to canonical RNA sequences yet Rho also has to act on non-canonical sites. Our genetic data suggest that the flexible connector region may modulate the transition between Rho’s inactive open and active closed states. We have identified substitutions that confer defects in Rho-dependent termination and are predicted to reduce connector flexibility (e.g., Gly→Asp). We hypothesize that the connector region is involved allosterically in binding to of divergent RNA sequences and is a potential target for factors that control Rho activity. Using molecular dynamic simulation software NAMD, we can develop a model of the connector region by mutating residues in silico. By modeling the closure of Rho mutants, we can assess the contributions of individual amino acid residues to Rho function. Quantitative analysis of root-mean-square deviation of atomic positions and inner pore diameter along with viewing the mechanism with VMD modeling software can give insights to how the selected substitutions affect ring closure. Additionally, elucidating the molecular details of Rho action will lead to understanding of other motor proteins that couple ATP hydrolysis to translocation on polymer substrates.
Research Project Title: Phenylalanyl-tRNA synthetase regulated quality control is linked to stress responses in Saccharomyces cerevisiae

Student Presenter: Amanda Kyle

Faculty Mentor: Michael Ibba

Faculty Mentor Department: Microbiology

Research Abstract: Compared to other essential cellular processes, translation is more error-prone, with an error rate of approximately 1 in $10^4$ codons. In translation, aminoacyl-tRNA synthetases (aaRSs) are enzymes which bind amino acids to cognate tRNAs. Some aaRSs have editing processes to prevent the misincorporation of structurally similar amino acids into proteins. Recently, it has been shown that phenylalanyl-tRNA synthetase (PheRS) quality control is important in the regulation of the general amino acid control (GAAC) pathway in Saccharomyces cerevisiae. To further explore how quality control affects S. cerevisiae, the growth of a PheRS editing deficient strain was compared to a wild-type (WT) strain in many conditions using phenotypic microarrays. Conditions where a difference in growth was observed were assigned to genes using previously established relationships between genes and chemicals. Many of these genes were associated with the target of rapamycin (TOR) and GAAC pathways. When grown in caffeine supplemented media, the PheRS editing deficient strain was more tolerant to caffeine than the WT strain. There is evidence linking mutations in the TOR pathway to differential growth in caffeine. To explore the relationship between PheRS quality control and the TOR pathway, growth of âˆ’TOR WT and âˆ’TOR PheRS editing deficient strains were compared in a chronological lifespan assay. Previously, prolonged chronological lifespans have been observed in âˆ’TOR yeast mutants. However, we observed that the âˆ’TOR mutation in the PheRS editing deficient strain had a decreased effect on chronological lifespan compared to the âˆ’TOR WT strain. This evidence suggests a relationship between PheRS regulated quality control and stress responses in S. cerevisiae. Since translation is a comparatively error-prone cellular process and there is evidence that mistranslation may be advantageous, it is important to understand how lacking quality control affects the cell.
Research Project Title: In vivo characterization of FACT complex proteins in C. elegans

Student Presenter: Aislinn Latham

Faculty Mentor: Helen Chamberlin

Faculty Mentor Department: Molecular Genetics

Research Abstract: FACT is a protein complex involved in transcriptional regulation, DNA replication, and DNA repair processes in all eukaryotic organisms. FACT allows transcriptional enzymes to access the DNA for transcription and eventual translation into proteins and it is highly conserved across species. However, there is limited knowledge about the FACT complex in multicellular organisms. The objective of this study is to discover more information about the composition and expression of the FACT complex and its role in multicellular organisms by tagging the genes that code for the FACT subunits in order to view the subsequent proteins in the nematode Caenorhabditis elegans. The complex is composed of two proteins: SSRP1 and SPT16. In C. elegans, two genes (hmg-3 and hmg-4) encode SSRP1 proteins, and one gene (spt-16) encodes SPT16. In order to determine the presence of the target subunits in C. elegans, sequences encoding a fluorescent tag were inserted into each gene using CRISPR-mediated genome editing. Using fluorescent microscopy conditions, the target proteins were visible in vivo, revealing the specific areas of the organism where the subunits were present. The location of the product in the cells of C. elegans differed between the HMG-3 and HMG-4 proteins. HMG-3 had somatic presence during the embryonic stage, but in later larval stages the product was confined to the germline. In contrast, HMG-4 and SPT-16 were present in the somatic and germ cells throughout the entire developmental process of the organisms. The FACT complex’s functions in DNA regulation and repair make it a potential target for future cancer and HIV research. Gaining information about the expression of the FACT complex’s proteins at different life stages in a multicellular eukaryote creates a base from which future research can draw. Future research may include interactions between subunits, and determining the number of each subunit protein in the FACT complex.
Research Project Title: Characterization and mapping of the required to maintain repression locus affecting paramutation in Zea mays

Student Presenter: Emily McCormic

Faculty Mentor: Jay Hollick

Faculty Mentor Department: Molecular Genetics

Research Abstract: Paramutation is a genetic phenomenon describing heritable changes caused by the interaction of a alleles at a specific locus. Such behavior is observed at multiple loci in different organisms, and defines patterns of inheritance that appear to defy the first law of Mendelian genetics. Paramutation at the Pl1-Rhoades (Pl1-Rh) allele of the purple plant1 (pl1) locus in the organism Zea mays results in meiotically heritable changes in gene regulation that are influenced by trans-homolog interactions. Plants with a fully-expressed Pl1-Rh allele exhibit dark anther coloration, while plants with a repressed paramutant derivative (denoted Plâ€™) lack anther coloration. The transcriptional and post-transcriptional repression of Pl1-Rh is facilitated by required to maintain repression (rmr) factors.

We previously reported on eight different rmr factors identified by mutations induced with ethyl methanesulfonate (EMS) pollen treatment. At least six of these factors are required for the biogenesis of 24-nucleotide sRNAs that may direct de novo cytosine methylation.

Genetic complementation tests and molecular mapping were used to determine that two recessive mutations, ems062986 and ems073240, define a novel locus provisionally designated rmr10. Results of genetic tests with the ems062986 mutation indicate that normal rmr10 function is required for the meiotic maintenance of Plâ€™ states but not for facilitating paramutations at the booster1 locus.

RNA-seq data and a bioinformatics pipeline are currently being used to identify likely candidate genes, and the effects of rmr10 function on pl1 RNA levels is being determined using qRT-PCR. Bulk low molecular weight RNA profiles will also be evaluated to characterize possible effects on small RNA accumulations. Results of these efforts expand our understanding of basic mechanisms controlling meiotically-heritable changes in gene regulation.
Research Project Title: Membrane localization of the F-BAR protein Rga7 depends on its F-BAR domain

Student Presenter: Shelby Naegele

Faculty Mentor: Jian-Qiu Wu

Faculty Mentor Department: Molecular Genetics

Research Abstract: F-BAR family proteins bind to phospholipids in the plasma membrane and are vital players in various cellular processes. However, the regulation and molecular mechanisms of their localization within the cell are not well understood. The purpose of this project is to study the localization of the F-BAR protein Rga7 in fission yeast cytokinesis. Rga7 contains an F-BAR domain at the N-terminus, and a Rho-GAP domain at the C-terminus. Normally, Rga7 localizes to the plasma membrane at the division site and the cell tips. The minimal region required for localization is Rga7 F-BAR domain. Recent studies from Wu lab have shown that proper cellular localization of Rga7 depends on its interaction with the coiled-coil protein Rng10. Rga7 cannot localize properly in the absence of Rng10. We also found that Rga7 F-BAR domain and Rng10 C-terminus interact directly and are able to bind phospholipids in vitro, with Rga7 binding preferentially to PI(4,5)P2. In this study, we created three lipid-binding mutants (PIP2, PIP2+Core, PIP2+Core+Tips) in Rga7 F-BAR domain using PCR-based gene targeting and examined the importance of membrane binding in Rga7 localization by confocal microscopy. Our data showed that PIP2+Core and PIP2+Core+Tips mutations abolished Rga7 localization on the plasma membrane at both the division site and the cell tips, whereas PIP2 mutants retained some localization at the division site. Overexpression of three Rga7 F-BAR mutants failed to rescue their localization on the plasma membrane. Surprisingly, loss of the membrane localization in Rga7 F-BAR mutants did not result in significantly more cell lysis compared to rga7Δ cells. Consistent with the important roles of PI(4,5)P2 on the plasma membrane, reducing the levels of PI(4,5)P2 significantly decreased Rga7 localization at the division site, but Rng10 localization was unaffected. Collectively, our data show that proper localization of the F-BAR protein Rga7 to the plasma membrane requires its F-BAR domain to bind the phospholipids and coiled-coil protein Rng10 simultaneously.
Research Project Title: Global analysis of cystic fibrosis and epigallocatechin gallate on CF mouse methylomes

Student Presenter: Alex Pan

Faculty Mentor: Pearly Yan

Faculty Mentor Department: Internal Medicine

Research Abstract: Introduction

Cystic fibrosis (CF) is a genetic disorder that perturbs respiration that leads to pulmonary infections. Previous studies have suggested that changes in DNA methylation, an epigenetic modification involving the addition of a 5-methyl group to the cytosine residue followed by guanine residue (CpG site), is associated with the development of CF. In a published study, the treatment of CF patients with the demethylating agent epigallocatechin gallate (EGCG) and cysteamine were shown to alleviate CF symptoms. However, the specific epigenetic effects of CF and EGCG treatment on the disease methylome are largely unknown.

Methods

Reduced representation bisulfite sequencing (RRBS) was used to quantify DNA methylation at single-base resolution in wild-type (WT), CF-, and EGCG-treated CF mice. The RRBS data were aligned to the bisulfite converted murine genome, and methylation signals were analyzed using the statistical tools in methylKit R-package to yield analyzed data such as PCA and differentially methylated cytosines (DMCs) between treatment groups. In addition, we utilized custom scripts to visualize RRBS data and to assist in the analysis of methylKit output.

Results

Of particular interest were DMCs localized in distal promoters. There were 5241 DMCs in WT vs CF and 3060 DMCs. These data revealed that CF mice had increased levels of DNA methylation in the proximal promoter regions in comparison to WT mice, and EGCG treatment on CF reduced the methylation levels to those found in the WT mice.

Conclusions

The CF and EGCG-treated CF methylomes showed a distinct pattern with global hypermethylation in CF samples and EGCG treatment was able to reverse this trend. Moving forward, we will focus on a gene-by-gene analysis of DMCs and CpGs to identify regions and genes that are preferentially perturbed by CF and EGCG-treatment.
Research Project Title: Yin and Yang mutants to study pre-synaptic Cre-loxP complex

Student Presenter: Devante Potter

Faculty Mentor: Mark Foster

Faculty Mentor Department: Chemistry and Biochemistry

Research Abstract: Cre recombinase is a site-specific DNA recombinase derived from bacteriophage P1, that is commonly used in biomedical research to recombine DNA molecules in a highly controlled manner, without the use of other cellular cofactors. To recombine its specific loxP DNA sequences, two pairs of Cre molecules assemble as a dimer of homodimers onto two loxP sites. Which are then brought together via protein-protein interactions to form a tetrameric Cre2-loxP. X-ray crystallographic studies have shown that the recombination pathway follows through this tetrameric synapse and a DNA four-way Holliday junction intermediate. However, little is understood about the intermediates leading to assembly of the recombination complex.

I seek to engineer two Cre mutants (termed "Yin" and "Yang"), totaling ten point mutations, that are incapable of forming the synaptic tetramer, but can assemble as a heterodimer on loxP, allowing formation of the assembly intermediate Cre2-loxP. Structural studies of these pre-synaptic complexes will lend insight to the study of dimer formation, DNA bending, and the thermodynamic contribution of each protomer to the binding of the asymmetric binding element between loxP sites.

To engineer Yin and Yang, I am using site directed mutagenesis by performing polymerase chain reactions (PCR) with complementary mutagenic DNA primers and a plasmid encoding the Cre gene as the starting template; at each step, the resulting mutagenized plasmid serves as the template for the next PCR reaction. Following completion of the construction of the genes encoding the Yin-Yang pair, the proteins will be expressed recombinantly in E. coli, purified and their complex on loxP will be characterized by electrophoretic mobility shift assays (EMSA). Thus far, I have succeeded in engineering Yin through site directed mutagenesis and the protein has been purified and expressed. Site directed mutagenesis to construct Yang is in progress.
Research Project Title: Characterization of DNA methylation in CHH contexts

Student Presenter: Whitney Powers

Faculty Mentor: Pearlyn Yan

Faculty Mentor Department: Internal Medicine

Research Abstract: Introduction

DNA methylation is an epigenetic modification which can repress transcription. Methylation in the human genome occurs at Cytosines (Cs) predominantly in CpG contexts (a Cytosine followed by a Guanine (G)), however, methylation can also occur in CHG and CHH contexts, where an H represents any base except for G. Single-base resolution methylation analysis can be achieved with reduced representation bisulfite sequencing (RRBS). Bisulfite conversion changes unmethylated Cs into Thymines (Ts). Methylated Cs are unaffected by this process. Since methylation primarily occurs at CpG sites, the amount of methylation in CHG and CHH contexts is traditionally used as an indicator for incomplete bisulfite conversion. Interestingly, we noted that RRBS data can sometimes harbor a non-significant amount of CHH methylation (~5%) and our analysis reveals that they tend to contain a sequence motif often found in centromeric regions.

Methods

RRBS data from OSU and from public data sets were analyzed. Regions containing methylated CHHs were investigated. Several samples were processed at the original 150 bp length, as well as trimmed to 75 bp. This allowed for the analysis of read length dependencies. Additionally, samples were filtered for high levels of methylated CHHs, as well as the presence of repetitive motifs. This allowed for the characterization of the locations of methylated CHHs.

Results

In RRBS data, CHH methylation appears to be distributed primarily in reads with multiple methylated CHHs. We found that much of CHH methylation is in reads with repetitive characteristics and shared a common motif often found in human centromeres. Interestingly, the alignment rate of these reads is highly dependent on the read length.

Conclusion

Our findings suggest that the alignment of these reads to the annotated portion of the human genome may be misleading, and the source of these reads may likely be the poorly annotated centromeric region. Furthermore, we show that the centromere contributes significant non-CpG methylation to RRBS data and by removing centromeric DNA prior to processing by library generation or by removing centromeric reads prior to alignment will increase the usability of CHH methylation as a surrogate of incomplete bisulfite conversion.
Research Project Title: A chemical crosslinking approach to elucidate the sub-unit arrangement of the essential tRNA deaminase from Trypanosoma brucei

Student Presenter: Amelia Staats

Faculty Mentor: Juan Alfonzo

Faculty Mentor Department: Microbiology

Research Abstract: The eukaryotic parasite, Trypanosoma brucei, is the causative agent of African Sleeping Sickness (Trypanosomiasis). According to the CDC, this disease threatens the lives of thousands of people in sub-Saharan Africa with a 100% mortality rate in untreated cases. Our laboratory studies transfer RNA (tRNA), an adaptor molecule which links the information encoded in the genome to protein synthesis. tRNAs receive a number of naturally occurring nucleotide chemical modifications, which are critical for translation; and therefore, are often essential to the viability of the parasite. Modifications may result in the change of one nucleotide to another; these belong to a sub-group of modifications collectively known as RNA editing. In T. brucei, certain tRNAs undergo editing events from A to I (adenosine to inosine) essential for viability, catalyzed by a heterodimeric enzyme called ADAT2/3 (adenosine deaminase acting on tRNA). This enzyme is made up of two distinct subunits, ADAT2 and ADAT3. Given their essentiality, it is important to understand the enzyme structures and function. The structure of ADAT2/3 has been difficult to elucidate partly due to the predictably highly dynamic nature of the N-terminal region of the ADAT3 subunit. In this study, we use chemical crosslinking to covalently bind the two subunits of ADAT2/3 together. Crosslinking dilutions will be made to determine the optimal concentration of chemical cross-linker to be used, ensuring that only one molecule of ADAT2 gets crosslinked to one molecule of ADAT3. This step is important to avoid crosslinks due to non-specific interactions that do not normally occur in the native enzyme and are therefore non-physiological. The properly crosslinked subunits will be analyzed by mass spectrometry to reveal key interactions between subunits, establishing their natural orientation. This analysis will allow us to determine the subunit conformation for this essential enzyme, which in turn will provide us with a better understanding of its function. In addition, to validate the results above, we will generate deletions to the N-terminal region of ADAT3 and determine how such deletions impact the ability of the enzyme to bind to the tRNA and catalyze the A to I reaction. Binding and activity assays will be conducted to assess how the cross-linked structure, as well as various mutants, affect enzyme function. Ultimately, elucidation of the deaminase structure is important to understand this unique modification pathway. In the long term, this information could potentially be exploited for the design of new anti-trypanosomal drugs and help combat this medically important parasite.
Research Project Title: Use of CRISPR to determine ligand effects on EGFR signaling

Student Presenter: Dan-Ho Tran

Faculty Mentor: Amanda Simcox

Faculty Mentor Department: Molecular Genetics

Research Abstract: Abstract

The Epidermal Growth Factor Receptor (EGFR) pathway is a complex and intensely studied signaling pathway that controls cell growth, proliferation, survival, and differentiation. The EGFR pathway is highly conserved in animals and altered pathway function can result in diseases like cancer. The signaling pathway is initiated by ligands (growth factors) that bind to the membranebound EGFR receptor and cause a signaling cascade into the nucleus through a common set of cellular proteins. However different ligands can result in different signaling outputs even though they use the same intracellular pathway. How these growth factors lead to different outcomes is an important question to study. In mammals, the EGFR pathway has many ligands (derived from eleven genes including EGF, TGF-alpha, epigen, and the neuregulins) and four receptors, whereas, in Drosophila there are only four ligands and one receptor. Due the relative simplicity of Drosophila it is easier to design an experiment to analyze downstream signaling triggered by a single ligand. In mammals this would be difficult because there are numerous ligands and these interact with multiple receptors generating great complexity. I will be focusing on the Drosophila ligands called Spitz (Spi) and Vein (Vn), which are members of different ligand families. Vn has a similar domain combination to the neuregulins and Spi is structurally similar to TGF-alpha ligands. Recent studies have led me to hypothesize that Spi will induce cell differentiation and Vn will cause cell proliferation. To test this hypothesis, cell lines expressing only Vn or only Spi will be generated using gene editing (CRISPR-Cas9). The other two Drosophila ligands share functional overlap with Spi and thus both can be deleted using CRISPR-Cas9. The cells will be used to evaluate differences in cellular phenotype and molecular signature (RNA-seq) following signaling initiated by single ligands (Vn or Spi). These results will be important for understanding how different responses are induced by distinct ligands using a common intracellular pathway.
Research Project Title: Identification of new players involved in creation of specific membrane domains in pollen of Arabidopsis thaliana

Student Presenter: Zachary Weber

Faculty Mentor: Anna Dobritsa

Faculty Mentor Department: Molecular Genetics

Research Abstract: Most pollen grains have, on their external surface, a complex arrangement of deposited materials. These deposits, known as exine, show species specific patterns and are often interrupted by distinct regions without deposits called apertures. Arabidopsis thaliana has three longitudinal apertures spaced equidistantly at the equator of the grain. The mechanisms by which sites for pollen apertures are selected and aperture formation occurs are still largely unknown. To identify potential members of the underlying pathway, we conducted a large screen of A. thaliana accessions to identify naturally occurring exine and aperture defects. From the screen, we found one accession with incompletely formed apertures. This defect was mapped using bulk segregate analysis and next generation sequencing to the gene encoding a kinase D6PKL3 and was confirmed via complementation and T-DNA insertion lines. We hypothesized that proteins affecting aperture development would localize to the positions of future apertures in developing pollen grains. We found that fluorescently-tagged D6PKL3 localized to the sites of future apertures on the interior of the plasma membrane during early pollen development. The structures of the D6 protein kinase-like family (D6PK) members predict conserved regions including phosphatidyl inositol phosphate (PIP) variant binding sites. PIP variants have previously been described to play a role in polarization of distinct membrane domains, and we suspected they may also play a role in specifying aperture domains on the plasma membrane. Lipid binding assays of D6PKL3 showed binding affinity for several PIP variants. To assay whether these PIP variants corresponded to developing apertures, we created fluorescently-tagged marker proteins known to interact with these PIP variants and tested their localization in developing pollen of A. thaliana. Two PIP variants, PI(4)P and PI(4,5)P2, were shown to localize to future aperture sites. D6PKL3 is only the second gene known to affect aperture formation in Arabidopsis. Its discovery and characterization are important to the formation of potential pathways in exine patterning and aperture formation. It’s structure and affinity for modified lipids also gives important direction for the identification of downstream and upstream interacting members.
Research Project Title: MetaPop: a metagenomic data analysis pipeline for investigating microbial population genetics

Student Presenter: Kenji Gerhardt

Faculty Mentor: Matthew Sullivan

Faculty Mentor Department: Microbiology

Research Abstract: Metagenomics, the study of genetic information in natural microbial populations, has revolutionized microbiology by elucidating the diversity of microbes and their interactions in complex communities. Although initially limited in such applications, recent improvements in genetic sequencing and computing technologies have made it possible to not only assess which populations are in a community, but also to study the diversity within these populations. Currently such efforts are largely manual. Here we sought to establish an analytical pipeline that enabled researchers to better study variation within populations from single nucleotide polymorphisms to population haplotypes in a way that could be applied to multiple samples capturing spatial and temporal variation in response to changes in their environment. This pipeline, which we call MetaPop, cleans and processes data to quantify population genetic metrics (codon bias, Tajima’s D, Pi and Theta, FST, and pN/pS) and identify haplotypes for abundant populations in deeply sequenced metagenomes, and provide metadata about each sample’s quality and breadth. MetaPop is mostly written in R, co-opts several popular metagenomic tools, and improves upon prior pipelines by (i) increasing the speed, breadth, and rigor of the metagenomic analyses being performed, (ii) empowering the user to process data locally or using a supercomputer, (iii) provides output in both tabular and figure formats to better enable exploration of patterns and trends, and (iv) scales linearly with data, so that the time taken to process larger datasets only increases linearly, instead of exponentially, so that MetaPop can handle datasets of any scale. By making population genetics more accessible and robustly applied to metagenomic datasets, we anticipate significant advances in establishing meaningful biological species definitions for microbial populations, as well as identifying and linking microbial microdiversity to ecological drivers and evolutionary processes.
Research Project Title: Identifying defects in midline axon guidance using fruit fly natural population lines

Student Presenter: Maya Gosztyla

Faculty Mentor: Mark Seeger

Faculty Mentor Department: Molecular Genetics

Research Abstract: The central nervous system (CNS) midline is an important choice point for many pathfinding axons during neural development. Previous studies have searched for novel regulators using mutagenesis experiments involving a few inbred laboratory strains of the fruit fly, Drosophila melanogaster. However, no studies thus far have attempted to utilize the polymorphic variation that exists in natural populations to study embryonic axon guidance at the CNS midline. This approach was recently enhanced by the creation of the D. melanogaster Genetic Reference Panel (DGRP), which consists of more than 200 isogenic, sequenced strains derived from an outbred population. In the present study, embryos from 142 DGRP strains were stained using one of two antibodies: BP102, which labels all axon pathways, or 1D4, which labels a subset of longitudinal axons that normally do not cross the CNS midline. We then selected a minimum of \( n = 5 \) embryos per strain for each antibody and scored for the presence of missing commissures or ectopic midline crossovers. We identified 39 strains where at least one embryo showed one or more defects in axon guidance. Of these, 18 showed only missing commissures, 16 showed only ectopic crossovers, and 5 showed both types of defects. Between these 39 strains, we observed considerable variation in the penetrance of the observed phenotypes, ranging from 5% to 65% of embryos showing a defect within a strain. These observations demonstrate that natural variation exists among genes influencing midline axon guidance in D. melanogaster. We are now repeating these experiments using the remaining DGRP strains. In addition, we are utilizing a sensitized genetic background to screen for additional strains with axon guidance defects. In the long-term, this research may provide insight into the complex network of ligands, receptors, and signaling molecules that regulate axon guidance.
Research Project Title: A genetic model of common, complex disease hints at genomic architecture

Student Presenter: Elizabeth McNamara

Faculty Mentor: Jennie Rowell

Faculty Mentor Department: College of Nursing

Research Abstract: Background: One of the greatest challenges facing scientists today is an understanding of genetic factors associated with the development of common, complex diseases such as diabetes, depression, and cancer. In addition to symptom burden (effecting quality of life), premature death, and disability caused by such diseases, the economic burden to society is vastly overwhelming. Last year, Major Depression alone cost nearly $211 Billion [USD] to US taxpayers. Yet, these diseases have been incredibly arduous to study due to the hundreds of genes that contribute very small effects that lead disease development. We suggest that an alternative model of complex diseases is needed to elucidate the causative genetic mechanisms. We exploit a naturally occurring clear phenotype (brindle color) present on a simplified genetic background (pet dogs) and analyze large-scale genomic variation (CNVs) effecting gene expression (epigenetics “DNA methylation).

Significance: Common, complex disease is a tremendous burden for patients, families, and clinicians alike. Elucidation of the genetic mechanisms leading to disease development would result in personalized therapies and improved patient outcomes.

Purpose: To identify large-scale genomic variations present in Brindle color dogs and determine if epigenetic regulation is leading to phenotype development.

Methods: Using a custom-designed aCGH to interrogate genomic structural variation, we analyzed 12 dogs of 3 different coat colors (Black, yellow, and brindle). Then, we performed genomewide DNA methylation analysis on a subset of 8 dogs to determine the effect genomic variation has on epigenetic silencing of gene expression.

Results: We identified a 67 Kb complex genetic variation (10 probes, p=0.001) that disrupts gene expression and is epigenetically silenced in certain skin cells producing pigment differences (p=0.04).

Conclusion: Brindle coat color in canines is a complex genetic mechanism involving structural changes leading to epigenetic effects. Identifying this mechanism provides the first tractability for understanding complex diseases and is particularly exciting as a model for identifying such features in human diseases.
Research Project Title: Exon skipping using an AAV-U7snRNA approach and its therapeutic implication for Duchenne Muscular Dystrophy.

Student Presenter: Daniel Lesman

Faculty Mentor: Nicolas Wein

Faculty Mentor Department: Center for Gene Therapy

Research Abstract: Duchenne Muscular Dystrophy (DMD) is a recessive X-linked degenerative muscle disorder affecting 1 / 5,200 males. DMD usually occurs due to mutations in the DMD gene that disrupt the reading frame. DMD encodes for dystrophin, a protein that links the F-actin cytoskeleton to the plasma membrane through its actin binding domains (ABD). Because of this, such domains were thought to be crucial for dystrophin function. However, recent evidence is challenging this dogma as proteins missing part of ABD1 were demonstrated to be highly functional. We have in the past developed a therapeutic vector inducing such protein lacking part of ABD1. Under this project we intend to develop another exon skipping vector inducing such proteins in order to potentially treat a sub population of DMD patients (~4% of patients). We developed a complex of proteins and antisense sequences (U7snRNA) mediating exon skipping. The advantage of our approach is that the antisense sequence is embedded, thereby protecting it from degradation and causing accumulation in the nucleus where splicing occurs. Moreover, when embedded into an Adeno-Associated Virus (AAV), these U7snRNAs can be permanently expressed. We transfected myotubes with a variety of plasmids to determine the best way to skip DMD exons. Our preliminary results support the ability of U7snRNA to mediate exon skipping. We are currently screening additional U7snRNA that mediates exon skipping. Cloning of the best candidates into a single AAV plasmid will then be performed to produce AAV. This AAV will be used in patient cell lines harboring mutation within exon encoding for the ABD1 domain. These results suggest that this U7snRNA vector offers a therapeutic approach for patient harboring mutations within exons encoding for ABD1 (~4% of patients), an area of the gene largely ignored by the current therapeutic approaches.
Research Project Title: Global local folding of the human transcriptome

Student Presenter: James Li

Faculty Mentor: Peter White

Faculty Mentor Department: Pediatrics

Research Abstract: Currently, disease-associated variants in humans are largely procured via their predicted functional impacts on proteins. Past in silico studies propose that selection in humans and mammals may have been shaped by mRNA secondary structure, but the direct impact of RNA folding on genetic diseases is not fully understood. Hence, a systematic whole transcriptome study of SNP (single nucleotide polymorphism) impacts on local RNA folding has yet to be undertaken. We aimed to (1) develop a cloud-based big data pipeline to generate RNA folding statistics for every possible polymorphism in the known human transcriptome (~0.5 billion variants), and (2) use population allele frequencies from 138,632 individuals, as well as mammalian conservation scores to see if SNPs causing large RNA disruptions were constrained, thus supporting our hypothesis that RNA stability and structure influence disease. First, all known RefSeq mRNA transcript sequences were retrieved and processed to generate 101 nucleotide flanking sequences for every position in each transcript. For every position, flanking sequences corresponding to each of the three possible alternate alleles were also generated and analyzed using our implementation of the ViennaRNA Package to obtain 10 RNA folding disruption metrics. SNPs were then joined with data from the Genome Aggregation Database, and ultimately annotated with SnpEff. Following this data compilation, we sorted the SNPs for each of their ten RNA folding metrics and binned these SNPs based on the percentiles of each of their metric values. Metric bins corresponding to higher RNA disruption values had a smaller fraction of SNPs with non-zero allele frequencies than metric bins corresponding to lower RNA disruption values. Analysis of mean and median GERP++ scores for each metric bin illustrated that positions of SNPs that were predicted to cause large RNA disruptions tended to have a deficit of mutations compared to a neutral substitution rate. The relationship of both constrained allele frequencies and GERP++ scores with increased RNA disruption metrics suggests that RNA folding disruptions may play a significant role in human health and disease.